

Electronic Supplementary Information

Characterisation

3-[10-Bromo-anthracen-9-yl]-acrylamide (3h)

mp- 258.0 °C; ν_{\max} (Nujol)/ cm^{-1} 3375 (N-H), 3200 (N-H), 1663 (C=O), 1600 (C=C); δ_{H} (400 MHz; d_6 -DMSO) 8.50 (2H, d, $^3J_{\text{HH}} = 8.8$ Hz, Ar-H), 8.26 (1H, d, $^3J_{\text{HH}} = 16.1$ Hz), 8.24 (2H, d, $^3J_{\text{HH}} = 8.8$ Hz, Ar-H), 7.76 (4H, ddd, $^3J_{\text{HH}} = 8.9$ Hz, $^3J_{\text{HH}} = 6.4$ Hz, $^4J_{\text{HH}} = 0.9$ Hz, Ar-H), 7.66 (4H, ddd, $^3J_{\text{HH}} = 8.9$ Hz, $^3J_{\text{HH}} = 6.4$ Hz, $^4J_{\text{HH}} = 0.9$ Hz, Ar-H), 7.41 (2H, s (broad), NH_2), 6.46 (1H, d, $^3J_{\text{HH}} = 16.1$ Hz, =CHCO), δ_{C} (d_6 -DMSO) 166.0, 141.5, 134.9, 129.5, 129.4, 129.3, 128.3, 128.6, 127.3, 126.3 m/z (EI) 326 [M⁺]. Found C, 62.89%; H, 3.74%; N, 4.38%. $\text{C}_{17}\text{H}_{12}\text{BrNO}$ requires C, 62.60%; H, 3.71%; N, 4.29%.

3-[10-Bromo-anthracen-9-yl]-acryloylmorpholine (3j)

mp- 278.2 °C; ν_{\max} (Nujol)/ cm^{-1} 1663 (C=O), 1600 (C=C); δ_{H} (400 MHz; d_6 -DMSO) 8.48 (2H, d, $^3J_{\text{HH}} = 8.6$ Hz, Ar-H), 8.31 (2H, d, $^3J_{\text{HH}} = 15.7$ Hz, Ar-CH=), 8.20 (2H, d, $^3J_{\text{HH}} = 8.6$ Hz, Ar-H), 7.64 (2H, ddd, $^3J_{\text{HH}} = 10.0$ Hz, $^3J_{\text{HH}} = 5.2$ Hz, $^4J_{\text{HH}} = 1.1$ Hz, Ar-H), 7.54 (2H, ddd, $^3J_{\text{HH}} = 10.0$ Hz, $^3J_{\text{HH}} = 5.2$ Hz, $^4J_{\text{HH}} = 1.1$ Hz, Ar-H), 6.93(2H, d, $^3J_{\text{HH}} = 15.7$ Hz, =CHCO), 3.54-3.60 (16H, m, $\text{OCH}_2\text{CH}_2\text{N}$). δ_{C} (d_6 -DMSO) 166.3, 145.4, 139.3, 138.2, 131.8, 127.7, 127.6, 126.7, 126.5, 121.8, 121.4, 126.3, 125.9, 43.0 30.5; m/z (EI) 393[M⁺]. Found C, 63.89%; H, 4.07%; N, 3.72%. $\text{C}_{21}\text{H}_{16}\text{NO}_2\text{Br}$ requires C, 64.12%; H, 4.07%; N, 3.56%.

(2E,2'E)-3,3'-(anthracene-9,10-diyl)bis(1-(piperidin-1-yl)prop-2-en-1-one) (4a)

mp- 294.4 °C; ν_{\max} (Nujol)/ cm^{-1} 1660 (C=O), 1600 (C=C); δ_{H} (400 MHz; CDCl_3) 8.53 (2H, d, $^3J_{\text{HH}} = 15.6$ Hz, Ar-CH=), 8.28 (4H, dd, $^3J_{\text{HH}} = 6.8$ Hz, $^4J_{\text{HH}} = 3.3$ Hz, Ar-H), 7.50 (4H, dd, $^3J_{\text{HH}} = 6.8$ Hz, $^4J_{\text{HH}} = 3.3$ Hz, Ar-H), 6.84 (2H, d, $^3J_{\text{HH}} = 15.6$ Hz, =CHCO), 3.77 (4H, m, NCH_aH_b), 3.55 (4H, m, NCH_aH_b), 1.70 (8H, m, $\text{NCH}_a\text{H}_b\text{CH}_2$), 1.61 (4H, m, $\text{NCH}_a\text{H}_b\text{CH}_2\text{CH}_2$); δ_{C} (CDCl_3)

164.1, 138.2, 132.0, 129.0, 128.7, 126.5, 126.2, 46.9, 43.2, 24.7; m/z (EI) 452[M⁺]. Found C, 80.01%; H, 6.95%; N, 5.70%. C₃₀H₃₂N₂O₂ requires C, 79.64%; H, 7.08%; N, 6.19%.

(2E,2'E)-3,3'-(anthracene-9,10-diyl)bis(N-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)acrylamide) (4i)

mp- 250.2 °C; ν_{\max} (Nujol)/cm⁻¹ 3400 (OH), 3250 (N-H), 1660 (C=O), 1600 (C=C); δ_{H} (400 MHz; d₆-DMSO) 9.04 (2H, s (broad), NH), 8.25 (2H, d, ³J_{HH} = 16.0 Hz, Ar-CH=), 8.23 (4H, dd, ³J_{HH} = 6.5 Hz, ⁴J_{HH} = 3.3 Hz, Ar-H), 7.59 (4H, dd, ³J_{HH} = 6.5 Hz, ⁴J_{HH} = 3.3 Hz, Ar-H), 6.68 (2H, d, ³J_{HH} = 16.0 Hz, =CHCO), 4.84 (6H, t, ³J_{HH} = 5.8 Hz, OH), 3.68 (12H, d, ³J_{HH} = 5.8 Hz, CH₂), δ_{C} (d₆-DMSO) 165.7, 135.6, 132.6, 131.7, 129.0, 126.8, 126.4, 63.5, 61.0; m/z (EI) 524[M⁺]. Found C, 63.89%; H, 5.92%; N, 5.69%. C₂₈H₃₂N₂O₈ requires C, 64.12%; H, 6.11%; N, 5.34%.

(2E,2'E)-3,3'-(anthracene-9,10-diyl)bis(1-morpholinoprop-2-en-1-one) (4j)

mp- 255 °C; ν_{\max} (Nujol)/cm⁻¹ 1660 (C=O), 1600 (C=C); δ_{H} (400 MHz; CDCl₃) 8.63 (2H, d, ³J_{HH} = 15.6 Hz, Ar-CH=), 8.26 (4H, dd, ³J_{HH} = 7.0 Hz, ⁴J_{HH} = 3.1 Hz, Ar-H), 7.52 (4H, dd, ³J_{HH} = 7.0 Hz, ⁴J_{HH} = 3.1 Hz, Ar-H), 6.79 (2H, d, ³J_{HH} = 15.6 Hz, =CHCO), 3.80 (16H, m (broad), NCH₂CH₂O), δ_{C} (d₆-DMSO) 164.9, 140.8, 132.0, 128.9, 126.0, 66.8, 46.2, 42.6; m/z (EI) 479[M+Na]. Found C, 73.86%; H, 5.99%; N, 5.97%. C₂₈H₂₈N₂O₄ requires C, 73.68%; H, 6.14%; N, 6.14%.

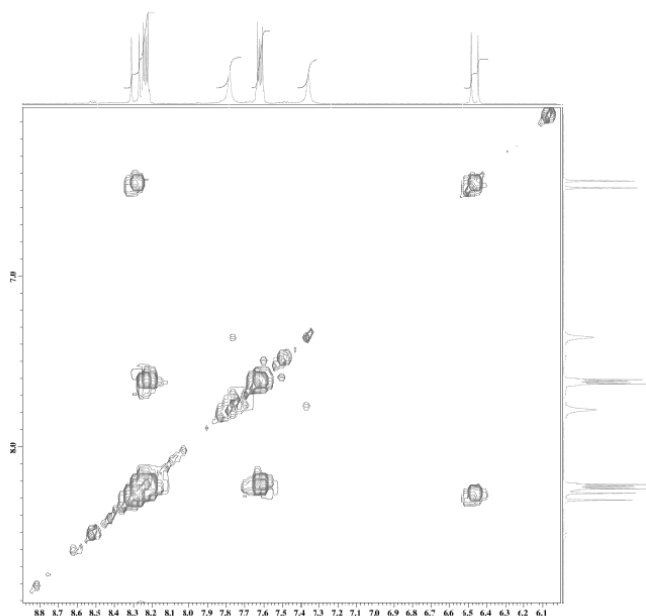
(2E,2'E)-3,3'-(anthracene-9,10-diyl)bis(N-phenylacrylamide) (4k)

mp- 368.5 °C; ν_{\max} (Nujol)/cm⁻¹ 3300 (N-H), 1663 (C=O), 1600 (C=C); δ_{H} (400 MHz; d₆-DMSO) 10.40(2H, s (broad), NH), 8.50 (2H, d, ³J_{HH} = 15.9 Hz, Ar-CH=), 8.23 (4H, dd, ³J_{HH} = 6.8 Hz, ⁴J_{HH} = 3.3 Hz, Ar-H), 7.79 (4H, d, ³J_{HH} = 7.6 Hz, Ar-H (Aniline)), 7.66 (4H, dd, ³J_{HH} = 6.8 Hz, ⁴J_{HH} = 3.3 Hz, Ar-H), 7.39 (4H, t, ³J_{HH} = 7.6 Hz, Ar-H (Aniline)), 7.13 (4H, t, ³J_{HH} = 7.6 Hz, Ar-H (Aniline)), 6.75(2H, d, ³J_{HH} = 15.9 Hz, =CHCO), δ_{C} (d₆-DMSO) 164.1, 155.9, 149.4,

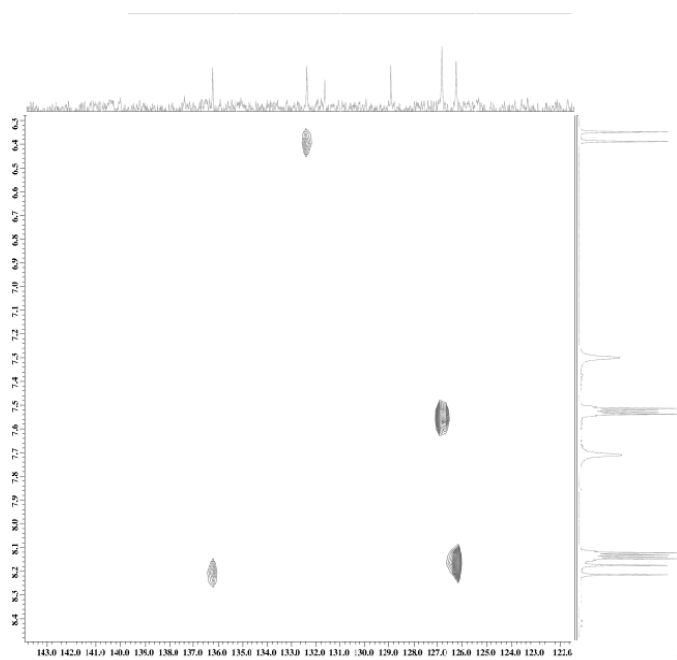
134.0, 132.5, 132.1, 129.7, 128.9, 127.1, 125.4 121.8, 121.4; m/z (EI) 468[M⁺]. Found C, 81.87%; H, 5.51%; N, 5.69%. C₃₂H₂₄N₂O₂ requires C, 82.05%; H, 5.13%; N, 5.98%.

(2E,2'E)-3,3'-(anthracene-9,10-diyl)bis(N-(1,1-dimethyl-3-oxobutyl)-acrylamide) (4I)

mp- 335.0 °C; ν_{\max} (Nujol)/cm⁻¹ 2932 (N-H), 1658 (C=O), 1625 (C=C); δ_{H} (400 MHz; d₆-DMSO) 8.26 (2H, d, ³J_{HH} = 15.8 Hz, Ar-CH=), 8.23 (4H, dd, ³J_{HH} = 6.8 Hz, ⁴J_{HH} = 3.3 Hz, Ar-H), 7.62 (4H, dd, ³J_{HH} = 6.8 Hz, ⁴J_{HH} = 3.3 Hz, Ar-H), 6.56 (2H, d, ³J_{HH} = 15.9 Hz, =CHCO), 3.09 (4H, s, CH₂), 2.13 (6H, s, CH₃CO), 1.42 (12H, s, C(CH₃)₂); δ_{C} (d₆-DMSO) 207.6, 164.4, 135.1, 132.9, 131.6, 128.9, 126.7, 126.2, 51.4, 40.1, 32.0, 27.6; m/z (EI) 547[M+2H₂O-2H].

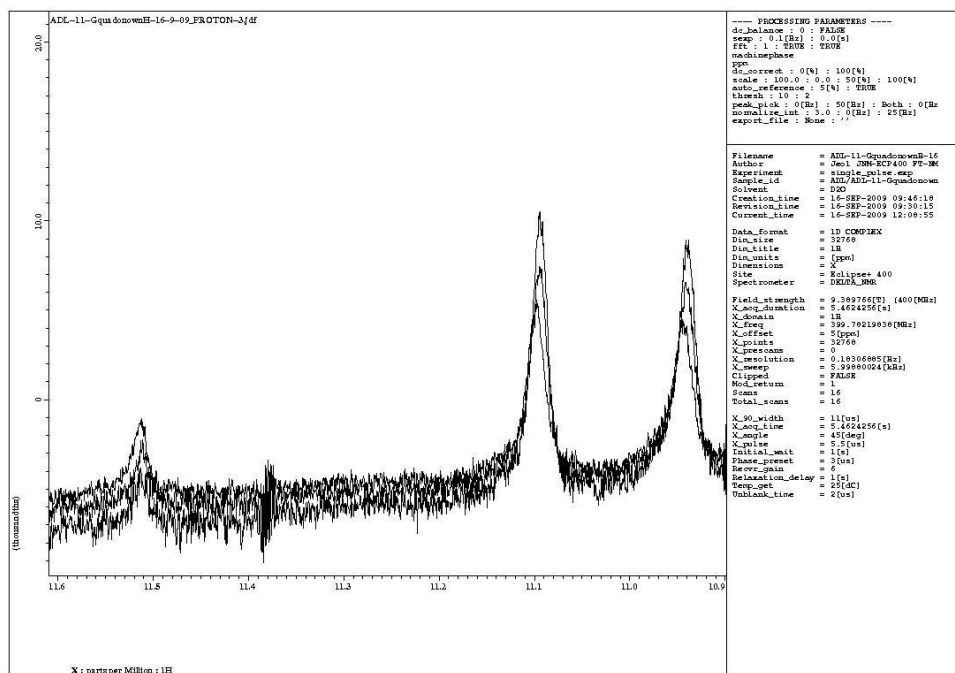


Expanded ¹H COSY NMR Spectrum for 4h



Expanded HETCOR NMR Spectrum for 4h

NMR



Expanded ^1H NMR showing growth of imine signal correlating to G-quadruplex formation at 11.6ppm 5, 10 and 15 minutes and the corresponding reduction in signal intensity for the imine signals at 11.1 and 10.95ppm, which have been shown to correlate to the monomeric TTGGGGT strand

Fluorescence displacement assay

The protocol of Mergny et al was followed:

A. De Cian, L. Lacroix, C. Douarre, N. Temime-Smaali, C. Trentesaux, C. Riou, J. Mergny, *Biochimie*, 2008, 90, 131.

A Thermofischer instrument was used to record the fluorescence spectra at 501 nm excitation wavelength. Data was collected between 510 and 750 nm (fluorescence area).

The slit width was set to 1nm

Preparation of the oligonucleotides

Quadruplexes and duplex from the 22AG were prepared by heating the oligonucleotides at 90°C for 5 mins in a 10 mM sodium cacodylate buffer (pH 7.3, 100 mM KCl). The solution was allowed to cool in the ice to favour intermolecular folding. For ds26 a self complementary strand was heated at 90°C for 5 min in a 10mM sodium cacodylate buffer (pH 7.3, 100mM KCl). concentrations of both of the DNA's were calculated by UV-VIS measurements at 260 nm λ maximum⁽¹⁴⁾

A Thermofischer instrument with cell system was used for the analysis. A 20°C temperature was kept constant 3ml cells were used for the each experiment .10mM sodium cacodylate buffer (pH 7.3 in 100mM KCl) was made up to a total volume of 3 ml.

Protocol for 22 AG

0.25 μ m of the 22AG was mixed with the 0.50 μ m of the thiazole orange. The blank was allowed to equilibrate for 10 minutes. Each ligand was added with the concentrations of 0.125, 0.25, 0.50, 1, 1.5, 2.5 μ m by using 3 min equilibration time after the fluorescence spectrum recorded. All experiments were repeated 5 times.

Protocol for ds26

0.25 μ m of the ds26 was mixed with the 0.75 μ m of the thiazole orange. The blank was allowed to equilibrate for 10 minutes. Each ligand was added with the concentrations of 0.125, 0.25, 0.50, 1, 1.5, and 2.5 μ m by using 3 min equilibration time after the fluorescence spectrum recorded. All experiments were repeated 5 times.

Calculation

The percentage of displacement was calculated by using following formula

$$\text{Percentage of displacement} = 100 - [(FA/FA_0) \times 100]$$

λ EX(Excitation)- 501 nm

FA --510-750 nm

FA₀ is the fluorescence of the thiazole orange bound to the DNA without added ligand. The graph between the percentage of displacement and concentration was plotted.

MTT cell viability assay protocol

The cells were treated with the drugs for 24 hours, 5 days and 10 day period. Cell counting was carried out a Haemocytometer and the cells analysed using sterilised numilon polystyrene TC treated 96 well plates obtained from Fischer. A LT-4000 microplate reader (Labtech International Ltd) was used to determine MTT conversion.

Chemicals

DMEM media, RPMI, L-glutamine-penicillin-streptomycin solution, trypan blue solution, trypsin and MTT (3-(4,5-dimethylthiazole-2-yl)-2,5 diphenyltetrazoliumbromide)dye were obtained from Sigma–Aldrich. Foetal bovine serum was obtained from Fisher Scientific.

Preparation of drugs

Drug 4h and 4j stock solutions were prepared by dissolving compounds in DMSO. The final concentration of DMSO in cultures was always less than 0.1% (v/v) and did not contribute to toxicity. For the MCF-7 cells, five different concentrations for each drug were used along with

a blank, 2.5 μ M, 12.5 μ M, 35 μ M, 50 μ M and 125 μ M. For the Caco-2 cells, 9 concentrations of 4h were used 0.05 μ M, 0.5 μ M, 1 μ M, 3 μ M, 5 μ M, 10 μ M, 30 μ M, 50 μ M and 100 μ M.

Cell culture

A breast cancer myeloma cell line MCF7 and Colorectal cell line Caco-2 were kindly provided by Dr Mark Carew and Dr Nick Freestone, Kingston University. The cell lines were cultured in DMEM media supplemented with penicillin G (100 U/ml), streptomycin (100 lg/ml), L-glutamine, and 10% foetal bovine serum at 37°C in a humidified atmosphere containing 5% CO₂. General experimental below is described for 4h.

Experimental:

DAY ONE: After Trypsinizing one culture flask, 5 ml of media was added to neutralise trypsin to make a suspension. This was then centrifuged for 1 min at 1000 rpm. The supernatant was removed and 10ml of media added followed by centrifuging again for 1 min at 1000 rpm. The supernatant was removed and resuspended in 5ml RPMI. Cells were counted using a haemocytometer after making (1:1) solution of 100 μ l cell suspension and 100 μ l trypan blue. Cells were then diluted and incubated overnight.

DAY TWO: Cells were treated with G-quadruplex ligand 4h. The media was removed and 100 μ l of drug was added to each well including the blank.

DAY THREE: After 24 hours MTT was added to each well. Include one row of wells with MTT but no drug which is called (control). The dye should be added aseptically and then incubated for 5 hours at 37°C. The media was removed, covered with tinfoil and analysed under the plate reader.

The same procedure was followed for 5 days and 10 days incubation period.

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Protocol for ds26

0.25 μM of the ds26 was mixed with the 0.75 μM of the thiazole orange. The blank was allowed to equilibrate for 10 minutes. Each ligand was added with the concentrations of 0.125, 0.25, 0.50, 1, 1.5, and 2.5 μM by using 3 min equilibration time after the fluorescence spectrum recorded. All experiments were repeated 5 times.

Calculation

The percentage of displacement was calculated by using following formula

$$\text{Percentage of displacement} = 100 - [(FA/FA_0) \times 100]$$

λ EX(Excitation)- 501 nm

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FA_0 is the fluorescence of the thiazole orange bound to the DNA without added ligand. The graph between the percentage of displacement and concentration was plotted.