Biaryl Polyamides as a New Class of DNA Quadruplex-Binding Ligands

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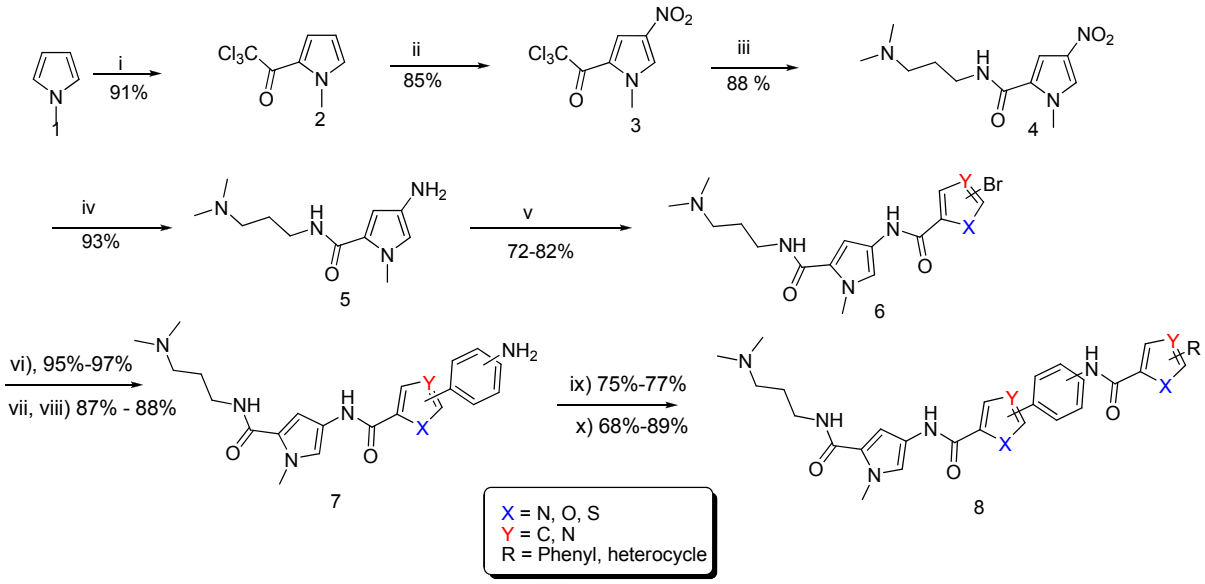
Supporting Information

General Methods

IR spectra were recorded on a Perkin-Elmer Spectrum 1000 FT IR Spectrometer. All spectra were recorded as solutions in deuterated dimethyl sulfoxide unless otherwise stated, and the chemical shifts are expressed in δ relative to tetramethylsilane (TMS) or residual DMSO as an internal standard. Coupling constants (*J*) are reported in hertz (Hz). The multiplicities of signals are denoted as s (singlet), bs (broad singlet), d (doublet), dd (double doublet), t (triplet), q (quartet) and m (multiplet). Low resolution mass spectra and reaction progress was monitored using a Waters Micromass ZQ system containing Waters 2996 PDA and Waters 2695 separation modules utilizing the electrospray ionization technique. Waters Micromass ZQ parameters used were: Capillary (kV), 3.38; Cone (V), 35; Extractor (V), 3.0; Source temperature (°C), 100; Desolvation Temperature (°C), 200; Cone flow rate (L/h), 50; Desolvation flow rate (L/h), 250. High-resolution mass spectra (HRMS) were obtained on a Thermo Navigator mass spectrometer coupled to LC using electrospray ionization (ES) and time-of-flight (TOF) mass spectrometry. Accurate molecular masses were determined using [Glu]-Fibrinopeptide B peptide ($[M+2H]^{2+}$ = 785.8426) as internal standard. All microwave heating was performed on Emry's Optimizer Personal Chemistry (Biotage AG). Excess solvents from reactions were evaporated using a Heidolph rotary evaporator under vacuum. Temperatures of reactions were controlled using an acetone/dry ice system unless otherwise specified. Thin Layer Chromatography (TLC) was performed on silica gel aluminium plates (Merck 60, F_{254}), and flash chromatography was carried out with silica gel (Merck 60, 230-400 mesh ASTM). After completion of reaction, reaction mixtures were initially passed through SCX-2 (silica-based sulfonic acid) cartridges which effectively trapped the basic compounds which were then eluted using 2M NH_3 in methanol. This ensured removal of excess reagents and minor impurities. Final purification involved either mass directed

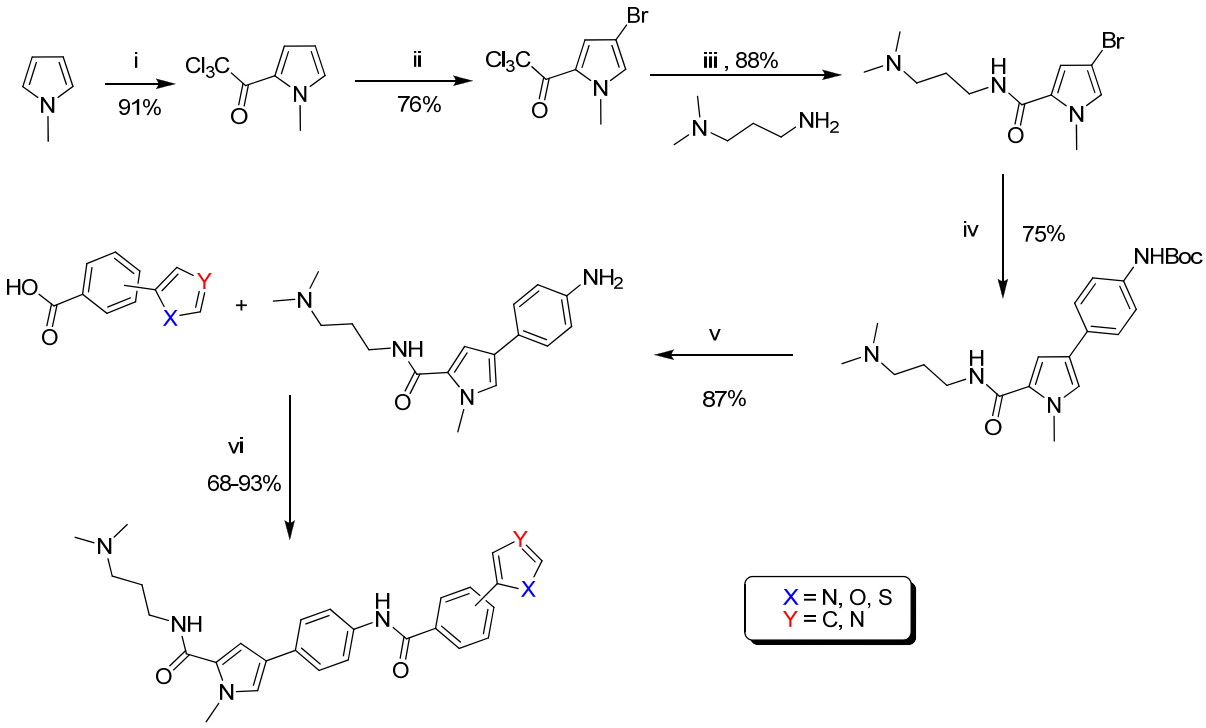
Water's preparative HPLC (Waters 2996 PDA detector, Waters 515 HPLC pump and Waters 2525 binary gradient module) or conventional column chromatography. Solvent from fractions containing pure compounds was evaporated using a VC3000D Genevac and then freeze dried using a Heto Lyolab 3000 freeze drier. All weighings were carried out using Sartorius high precision balances. All parallel reactions were carried out in a Radleys^{TM} Greenhouse parallel synthesiser



Reagents and conditions

i) Trichloroacetyl chloride, THF, RT, 4 h ii) Conc. HNO_3, Acetic anhydride, -5^0 C, 3 h iii) N,N- dimethylaminopropyl amine, dry THF, RT, 6 h iv) H_2, Pd/C, 4 h v) 5-bromoheterocyclic acid, DIC, HOBt, DCM, RT, 16 h vi) 4-aminoboronic acid, $(PPh_3)_4Pd$, K_2CO_3, Ethanol : Tolune: Water - 9:3:1, MW, 12-23 Minutes vii) 3-nitroboronic acid, $(PPh_3)_4Pd$, K_2CO_3, Ethanol : Tolune: Water - 9:3:1, MW, 8-15 Minutes; viii) H_2, Pd/C, EtOAC, 4 h ix) heterocyclic carbonyl chloride, Dry DMF, RT, 2 h x) heterocyclic carboxylic acid, DIC, HOBT, DCM, RT, 16 h

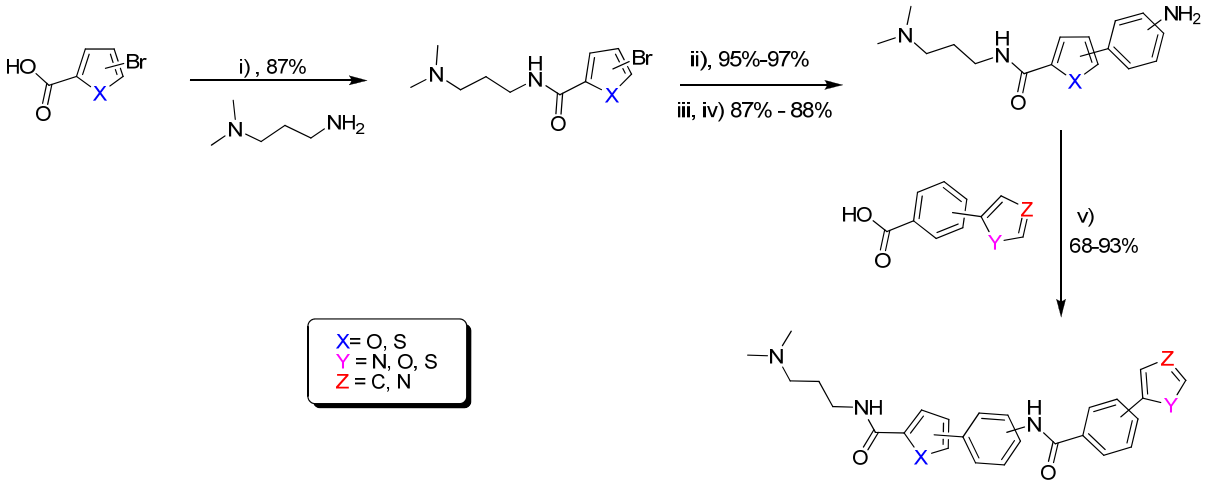
Scheme S1 : The synthesis of biaryl polyamides (Motif-1)



Reagents and conditions

i) Trichloroacetyl chloride, THF, RT, 4 h ii) NBS, Dry THF, -10 0C for 2 h, RT for 4 h iii) Dry THF, RT, 6 h,
iv) 4-(tert-butoxycarbonylamino)phenylboronic acid, (PPh3)4Pd, K2CO3, Ethanol : Tolune: Water - 9:3:1, MW, 12 Minute
v) 4M HCl in dioxane, 3h, room temperature vi) DIC, HOBT, DCM, RT, 16 h

Scheme S2 : The synthesis of biaryl polyamides (Motif-2) - 1



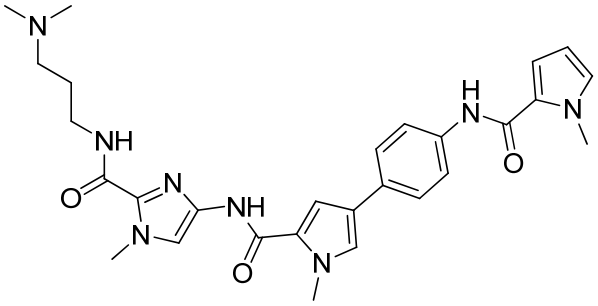
Reagents and conditions

i) DIC, HOBT, DCM, RT, 16 h ii) 4-aminoboronic acid, $(PPh_3)_4Pd$, K_2CO_3, Ethanol : Tolune: Water - 9:3:1, MW, 12-23 Minutes
iii) 3-nitroboronic acid, $(PPh_3)_4Pd$, K_2CO_3, Ethanol : Tolune: Water - 9:3:1, MW, 8-15 Minutes; iv) H_2, Pd/C, EtOAC, 4 h
v) DIC, HOBT, DCM, RT, 16 h

Scheme S3 : The synthesis of biaryl polyamides (Motif-2) - 2

Characterization Data for Ligands 1-6

1



N-(3-(dimethylamino)propyl)-1-methyl-4-(1-methyl-4-(4-(1-methyl-1H-pyrrole-2-carboxamido)phenyl)-1H-pyrrole-2-carboxamido)-1H-imidazole-2-carboxamide :

A cream solid. R_f 0.36 (DCM:MeOH:2 M NH_3 in MeOH - 8:1:1), ν_{max} (solid), 3281, 2358, 2186, 1589, 1520, 1463, 1430, 1360, 1278, 1104, 933, 816, 659; δ_H (400 MHz, DMSO); 10.31 (1H, s, NH), 9.75 (1H, s, NH), 8.03 (1H, t, *J* = 6, NH), 7.71 (2H, d, *J* = 8.8, 2ArH), 7.53 (1H, s, PyH), 7.52 (1H, s, ImH), 7.48 (2H, d, *J* = 8.8, 2ArH), 7.42 (1H, s, PyH), 7.04 (2H, m, 2PyH), 6.1 (1H, t, *J* = 4, PyH), 3.94 (3H, s, NCH_3), 3.92 (3H, s, NCH_3), 3.89 (3H, s, NCH_3), 3.27 (2H, t, *J* = 6.4, CH_2), 2.26 (2H, t, *J* = 7.2, CH_2), 2.15 (6H, s, 2N-CH_3), 1.64 (2H, m, CH_2); δ_C (100 MH, DMSO): 161.2 (C=O), 159.7(C=O), 158.5(C=O), 156.2 (Im-C), 149.8 (Im-C) , 137.0 (Im-C), 136.1 (Ar-C), 135.1 (Py-C), 134.1 (Py-C), 129.6 (Ar-C), 128.7 (Py-C), 127.6 (Py-C), 125.3 (2Ar-C), 124.4 (Py-C), 122.1 (Ar-C), 120.4 (Ar-C), 113.5 (Py-C), 110.7 (Py-C), 106.7 (Py-C), 56.8 (NCH_2), 45.1($2NCH_3$), 40.2 (CH_2) 36.9 (NCH_3), 36.5 (NCH_3), 36.3 (NCH_3), 34.84 (NCH_3), 27.1 (CH_2); *m/z* (+EI) calc. for $C_{28}H_{34}N_8O_3$ $(M+H)^+$ 531.2825, found 531.2819 $(M+H)^+$.

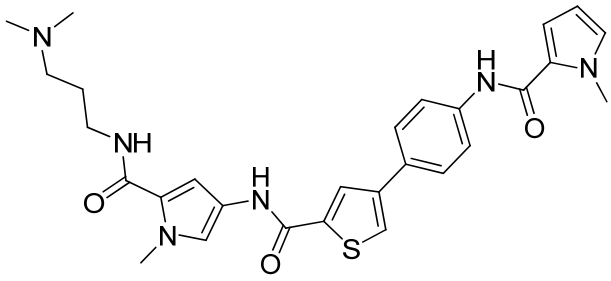
Note :

Py = Pyrrole

Im = Imidazole

Ar = Aryl/Phenyl

2



[N-(5-(3-(dimethylamino)propylcarbamoyl)-1-methyl-1H-pyrrol-3-yl)-2-(4-(1-methyl-1H-pyrrole-2-carboxamido)phenyl)-thiophene-2-carboxamide]: A cream solid. R_f 0.44 (DCM:MeOH:2 M NH_3 in MeOH - 8:1:1), ν_{max} (solid), 3260, 2179, 1995, 1634, 1589, 1515, 1415, 1328, 1287, 1249, 1105, 873, 835, 774, 735; δ_H (400 MHz, DMSO); 10.33(1H, s, NH), 9.84 (1H, s, NH), 8.32 (1H, d, *J* = 0.8 Hz, ThioH), 8.12 (1H, t, *J* = 5.6, 1H, s, NH), 8.03 (1H, d, *J* = 0.8, ThioH), 7.83 (2H, d, *J* = 8.8, 2ArH), 7.68 (2H, d, *J* = 8.8, 2ArH), 7.23 (1H, d, *J* = 1.6, PyH), 7.06 (1H, dd, *J* = 4.0, 1.6, PyH), 7.02 (1H, t, *J* = 2.0, PyH), 6.85 (1H, d, *J* = 1.6, PyH), 6.11 (1H, dd, *J* = 3.6, 1.2 PyH), 3.90 (3H, s, NCH_3), 3.83 (3H, s, NCH_3), 3.22 (2H, q, *J* = 6.8, CH_2), 2.35 (2H, t, *J* = 6.8, CH_2), 2.22 (6H, s, $2NCH_3$), 1.65 (2H, m, CH_2); δ_C (100 MH, DMSO): δ 161.1 (C=O), 159.8 (C=O), 158.2 (C=O), 141.6 (Thio-C), 140.6 (Thio-C), 138.9 (Thio-C), 137.2 (Ar-C), 129.4 (Ar-C), 129.0 (Py-C), 126.5 (Thio-C), 126.0 (2Ar-C), 125.3 (Py-C), 124.5 (Py-C), 123.4 (Py-C), 121.4 (Py-C), 120.2 (Ar-C), 118.0 (Ar-C), 113.8 (Py-C), 106.8 (Py-C), 103.9 (Py-C), 56.9 (NCH_2), 44.9 ($2NCH_3$), 40.1 (NCH_2), 36.3(NCH_3) , 35.9 (NCH_3), 26.9 (CH_2); *m/z* (+EI) calc. for $C_{28}H_{32}N_6O_3S$ $(M+H)^+$ 533.2329, found 533.2319 $(M+H)^+$.

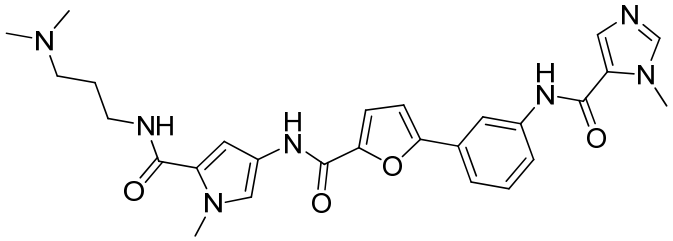
Note :

Py = Pyrrole

Thio = Thiophene

Ar = Aryl/Phenyl

3



[N-(5-(3-(dimethylamino)propylcarbamoyl)-1-methyl-1H-pyrrol-3-yl)-2-(3-(1-methyl-1H-imidazole-5-carboxamido)phenyl)-furan-5-carboxamide] : A cream solid. R_f 0.31 (DCM:MeOH:2 M NH_3 in MeOH - 8:1:1), ν_{max} (solid), 3085, 2193, 1639, 1620, 1538, 1434, 1354, 1179, 1028, 840, 763; δ_H (400 MHz, DMSO); 10.35 (1H, s, NH), 10.26 (1H, s, NH), 8.31 (1H, s, ImH), 8.11 (1H,t, *J* = 5.2 Hz, NH), 7.85 (1H, d, *J* = 8.4, ArH), 7.69 (1H, d, *J* = 7.6, ArH), 7.47 (2H, m, 2ArH), 7.32 (1H, d, *J* = 3.6, FuH), 7.25 (1H, d, *J* = 2.0, ImH), 7.12 (1H, d, *J* = 0.8, PyH), 7.06 (1H, d, *J* = 3.2, FuH), 6.89 (1H, d, *J* = 2.0, PyH), 4.03 (3H, s, NCH_3), 3.84 (3H, s, NCH_3), 3.20 (2H, q, *J* = 6.8, CH_2), 2.33 (2H, t, *J* = 7.2, CH_2), 2.20 (6H, s, $2NCH_3$), 1.64 (2H, m, CH_2); δ_C (100 MHz, DMSO): δ 161.1(C=O), 157.4 (C=O), 154.7 (C=O), 154.5 (Fu-C), 147.0 (Im-C), 138.9 (Fu-C), 138.4 (Im-C), 129.8 (Ar-C), 129.3(Ar-C), 127.3 (Ar-C), 127.1 (Py-C), 123.4 (Ar-C), 121.2 (Py-C), 120.4 (Py-C), 119.9 (Ar-C), 118.2 (Py-C), 116.1 (Ar-C), 115.8 (Ar-C), 107.8 (Fu-C), 104.2 (Fu-C), 57.0 (NCH_2), 45.1($2NCH_3$) , 40.3 1 (NCH_2), 37.1(NCH_3), 36.0 (NCH_3), 27.1 (CH_2) ; *m/z* (+EI) calc. for $C_{27}H_{31}N_7O_4$ $(M+H)^+$ 518.2510, found 518.2508 $(M+H)^+$.

Note :

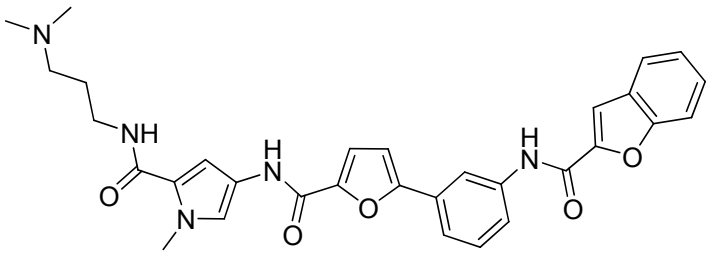
Py = Pyrrole

Im = Imidazole

Fu = Furan

Ar = Aryl/Phenyl

4



[4-(5-(3-(benzofuran-2-carboxamido)phenyl)furan-2-carboxamido)-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide] : A cream solid. R_f 0.39 (DCM:MeOH:2 M NH_3 in MeOH - 8:1:1) ν_{max} (solid), 3265, 2359, 2014, 1629, 1588, 1539, 1434, 1279, 1010, 747, 654; δ_H (400 MHz, DMSO); 10.77 (1H, s, NH), 10.34 (1H, s, NH), 8.30 (1H, s, ArH), 8.11 (1H, t, J = 5.6 Hz, NH), 7.87 (3H, m, 3ArH), 7.74 (2H, t, J = 8.4, 2ArH), 7.53 (1H, s, FuH), 7.52 (1H, m, ArH) 7.39 (1H, t, J = 7.2, ArH), 7.33 (1H, d, J = 3.2, FuH), 7.26 (1H, d, J = 1.6, PyH), 7.09 (1H, d, J = 3.6, FuH), 6.88 (1H, d, J = 1.6, PyH), 3.84 (3H, s, NCH_3), 3.20 (2H, q, J = 6, CH_2), 2.25 (2H, t, J = 7.2, CH_2), 2.16 (6H, s, $2NCH_3$), 1.64 (2H, m, CH_2); δ_C (100 MHz, DMSO): δ 165.6 (C=O), 161.1 (C=O), 156.8 (C=O), 154.7(BnFu-C), 154.4 (Fu-C), 148.6 (BnFu-C), 147.10(Fu-C), 138.9 (Ar-C), 129.8 (Ar-C), 129.3 (Pu-C), 127.2 (Ar-C), 127.1 (BnFu-C), 123.9 (Py-C), 123.4 (Py-C), 122.9 (BnFu-C), 121.2 (BnFu-C), 120.9 (Ar-C), 120.3 (Ar-C), 118.1 (BnFu-C), 116.6 (Py-C), 115.7 (Ar-C), 111.9 (BnFu-C), 110.77 (BnFu-C), 107.8 (Fu-C), 104.19 (Fu-C), 56.9 (NCH_2), 45.0 ($2NCH_3$), 40.1 (CH_2), 37.0 (N-CH_3), 27.0 (CH_2) ; *m/z* (+EI) calc. for $C_{31}H_{31}N_5O_5$ $(M+H)^+$ 554.2396, found 554.2398 $(M+H)^+$.

Note :

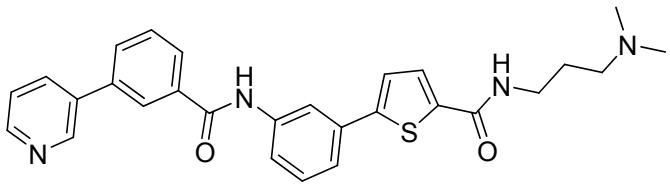
Py = Pyrrole

BnFu = Benzofuran

Fu = Furan

Ar = Aryl/Phenyl

5



[5-(3-(3-(pyridin-3-yl)benzamido)phenyl)-N-(3-(dimethylamino)propyl)thiophene-2-carboxamide]: A cream solid. R_f 0.48 (DCM:MeOH:2 M NH_3 in MeOH - 8:1:1), ν_{max} (solid), 3268, 2137, 1601, 1584, 1545, 1486, 1456, 1305, 1253, 875, 853, 781, 741; δ_H (400 MHz, DMSO,) 10.48 (1H, s, NH), 9.03 (1H, d, *J* = 1.80 Hz, PyrdH), 8.64 (1H, dd, *J* = 4.8, 1.6, PyrdH), 8.61 (1H, d, *J* = 5.6, PyrdH), 8.33 (1H, t, *J* = 5.6, NH), 8.27 (1H, s, ArH), 8.20 (1H, t, *J* = 1.6, ArH), 8.04 (1H, dt, *J* = 8.0, 1.6 ArH), 7.98 (1H, dt, *J* = 8.0, 1.6, ArH), 7.82 (1H, dt, *J* = 7.6, 2, ArH), 7.75(1H, d, *J* = 4, ThioH), 7.70 (1H, t, *J* = 7.6, ArH), 7.56 (1H, ddd, *J* = 8.0, 4.8, 0.8, ArH), 7.51 (1H, d, *J* = 4, ThioH), 7.48 (1H, m, PyrdH) 7.46 (1H, t, *J* = 7.6, ArH), 3.28 (2H, t, *J* = 6.7, CH_2), 2.45 (2H, t, *J* = 6.8, CH_2), 2.27 (6H, s, $2NCH_3$), 1.71 (2H, m, CH_2), δ_C (100 MHz, DMSO): δ 165.3 (C=O), 160.9 (C=O), 148.9 (Pyrd-C), 147.9 (Pyrd-C), 147.1 (Thio-C), 139.8 (Thio-C), 139.1 (Ar-C), 137.2 (Ar-C), 135.5 (Thio-C), 134.9 (Ar-C), 134.3 (Pyrd-C), 133.6 (Pyrd-C), 133.5 (Ar-C), 129.6 (Ar-C), 129.3 (Ar-C), 128.9 (Ar-C), 128.7 (Thio-C), 127.5 (Ar-C), 126.0 (Ar-C), 123.9 (Pyrd-C), 120.9 (Ar-C), 120.2 (Ar-C), 117.4 (Ar-C), 56.2 (NCH_2), 44.4 ($2NCH_3$), 39.7 (CH_2), 26.5 (CH_2); ; *m/z* (+EI) calc. for $C_{28}H_{28}N_4O_2S$ $(M+H)^+$ 485.2006, found 485.1996 $(M+H)^+$.

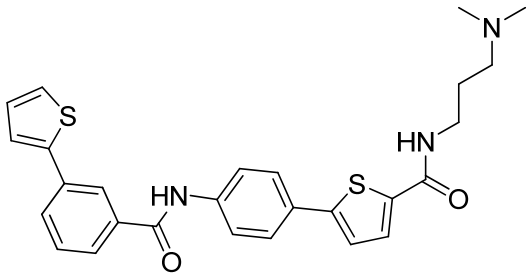
Note :

Pyrd = Pyridyl

Thio = Thiophene

Ar = Aryl/Phenyl

6



[5-(4-(3-(thiophen-2-yl)benzamido)phenyl)-N-(3-(dimethylamino)propyl)thiophene-2-carboxamide] : A cream solid. R_f 0.53 (DCM:MeOH:2 M NH_3 in MeOH - 8:1:1), δ_H (400 MHz, DMSO,); 10.51 (1H, s, NH), 8.53 (1H, t, *J* = 5.6 Hz, NH), 8.20 (1H, t, *J* = 1.6, ArH), 7.89 (4H, d, , *J* = 8.8, 4ArH), 7.72 (3H, m, 3ArH), 7.66 (1H, dd, *J* = 3.6, 1.2, ThioH), 7.63 (1H, dd, *J* = 5.2, 1.2, ThioH), 7.60 (1H, t, *J* = 4.0, ThioH), 7.49 (1H, d, *J* = 3.6, ThioH), 7.20 (1H, dd, *J* = 3.2, 1.2, ThioH), 3.26 (2H, q, *J* = 6.4, CH_2), 2.28 (2H, t, *J* = 7.2, CH_2), 2.16 (6H, s), 1.66 (2H, m, CH_2), δ_C (100 MHz, DMSO): δ 165.3 (C=O), 160.9(C=O), 147.0 (Thio-C), 142.4 (Thio-C), 139.3 (Ar-C), 138.5 (Thio-C), 135.6 (Thio-C), 133.9 (Ar-C), 129.3 (Ar-C), 128.8 (Thio-C), 128.6 (Thio-C), 128.4 (Ar-C), 126.7 (Thio-C), 126.3 (Thio-C), 125.9 (2Ar-C), 124.6 (Ar-C), 124.4 (Ar-C), 123.5 (Ar-C), 120.7 (2Ar-C), 56.8 (NCH_2), 45.0 ($2NCH_3$), 40.2 (CH_2), 27.1 (CH_2); *m/z* (+EI) calc. for $C_{27}H_{27}N_3O_2S_2$ $(M+H)^+$ 490.1617, found 490.1627 $(M+H)^+$.

Note :

Thio = Thiophene

Ar = Aryl/Phenyl

Fluorescent Resonance Energy Transfer (FRET)-Based DNA Melting Assay

The fluorescence tagged *Seq-3* (Eurogentec, UK) stock solution in water (20 μM) was diluted to 400 nM using FRET buffer (50 mM potassium cacodylate, pH 7.4) and annealed by heating at 85ºC for 5 minutes followed by cooling to room temperature over 5 hours. Ligand solutions were prepared in concentrations double that required for final solutions. Dilutions from the initial 10 mM DMSO stock solution were performed using FRET buffer. 50 μL of annealed DNA and 50 μL of sample solution were added to each well of a 96-well plate (MJ Research, Waltham, MA) and processed in a DNA Engine Opticon (MJ Research). Fluorescence readings were taken at intervals of 0.5°C over the range 30-100°C, with a constant temperature maintained for 30 seconds prior to each reading. The incident radiation was 450-495 nm and detection was conducted at 515-545 nm. The raw data were imported into the program Origin (version 7.0, OringinLab Corp.), and the graphs were smoothed using a 10-point running average and subsequently normalised. Determination of melting temperatures was performed by obtaining values at the maxima of the first derivative of the smoothed melting curves using a script. The difference between the melting temperature of each sample and that of the blank (ΔTm) was used for comparative purposes. The ΔTm values were plotted against concentrations of the ligands using Sigmaplot 11.0 to obtain the melting curves presented here.

Table S1 : DNA G-Quadruplex Stabilization by 1-6 Through FRET Melting Experiments

Code	ΔTm (^{0}C)											
	F21T				Ckit1				Ckit2			
	1 μM	2 μM	5 μM	10 μM	1 μM	2 μM	5 μM	10 μM	1 μM	2 μM	5 μM	10 μM
1	8.8	12.7	19.5	23.1	6.1	7.7	12.9	22.1	3.2	8.6	19.1	27.8
2	2.4	4.6	8.2	13.5	2.5	2.6	8.42	18.4	3.5	4.4	12.9	22.2
3	11.8	14.1	19.6	23.1	6.0	6.9	15.0	19.8	5.6	8.7	> 35	> 35
4	6.8	12	18.3	23.2	4.8	5.8	13.1	22.7	4.7	5.4	10.9	24.9
5	5.9	8.9	16.3	18.5	4.3	6.4	10.3	17.7	2.4	3.2	9.5	16.3
6	1.0	1.5	12.1	25.1	4.4	8.7	12.1	33.8	4.6	8.7	17.9	30.4

Table S2 : Selectivity of 1-6 For Quadruplex Sequences Compared to Duplex at 1 µM Concentration.

Ligand	ΔT_m Q at 1µM conc. (^{0}C)				Selectivity ΔTmQ/ ΔTmD		
	HT4	c-kit1	c-kit2	Duplex	HT4	c-kit1	c-kit2
1	8.8	6.1	3.2	0.4	22	15.25	8
2	2.4	2.5	3.5	0.2	12	12.5	17.5
3	11.8	6.0	5.6	0.0	∞	∞	∞
4	6.8	4.8	4.7	0.3	22.6	16	16
5	5.9	4.3	2.4	0.1	59	43	24
6	1.0	4.4	4.6	0.0	∞	∞	∞
Distamycin	0.6	0.3	0.4	11.5	0.05	0.025	0.034

FRET Melting Curves for Ligands 1-6

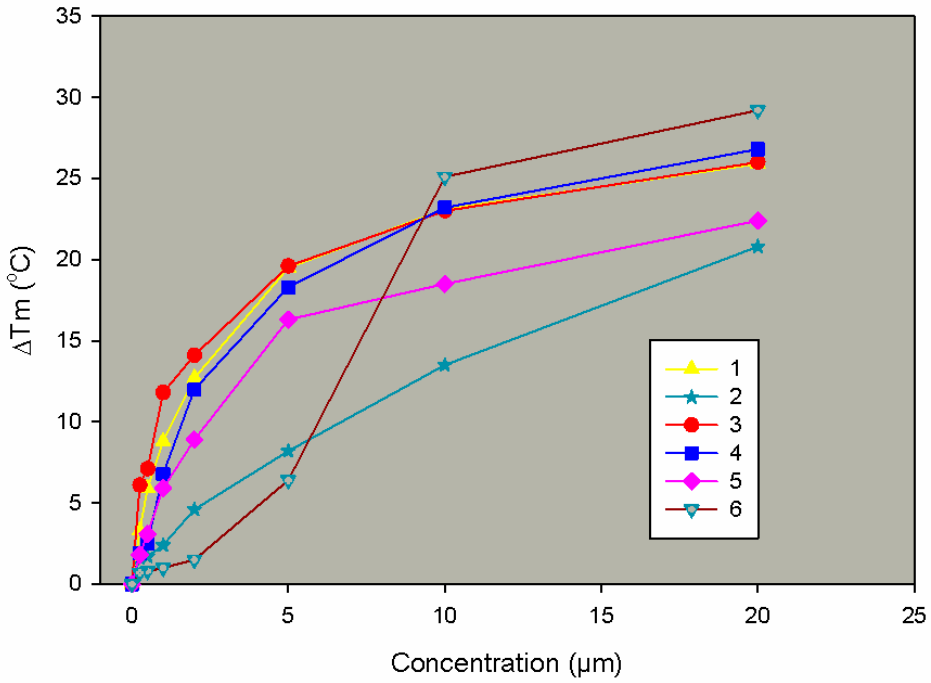


Figure S1 : FRET melting curves for ligands 1-6 against F21T (HT4)

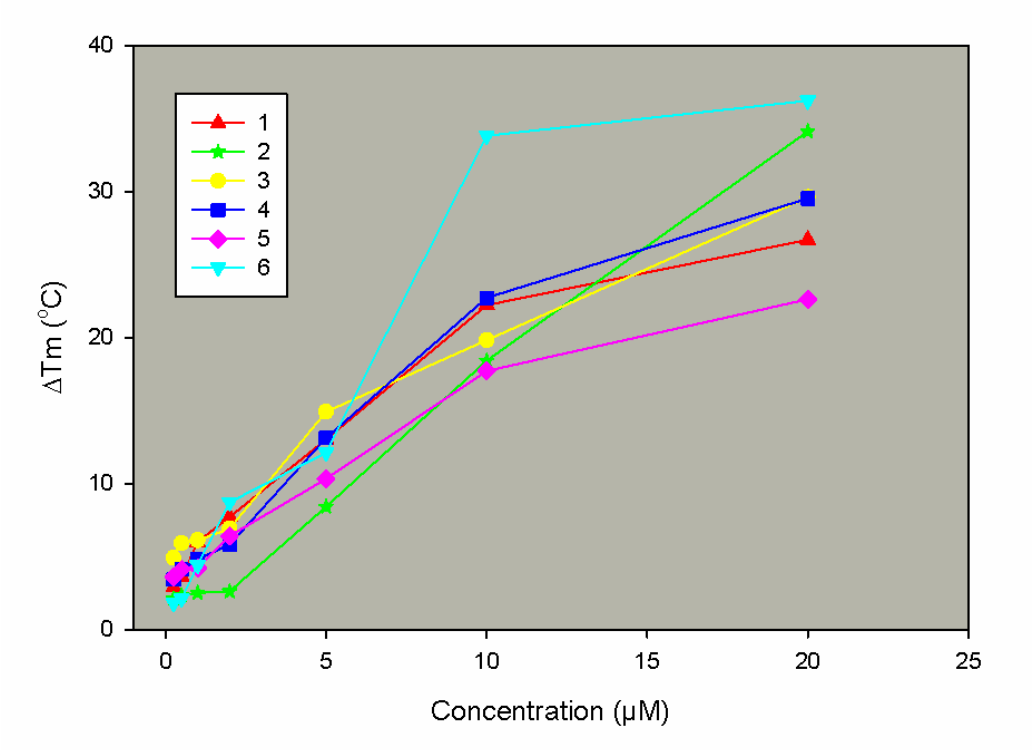


Figure S2 : FRET melting curves for ligands 1-6 against CKit1

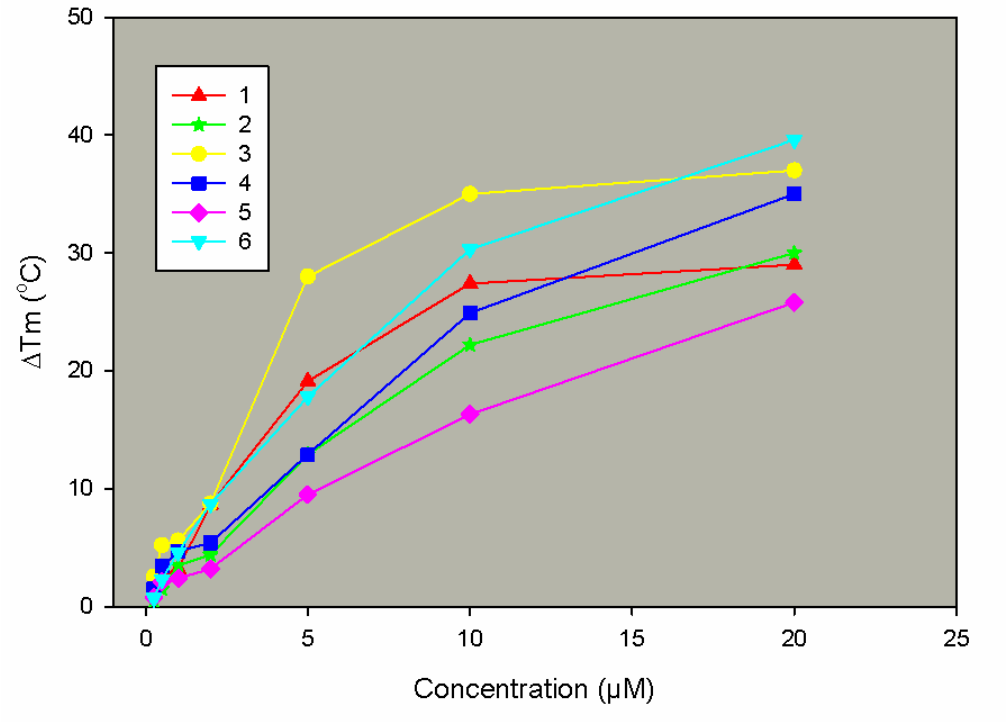


Figure S3: FRET melting curves for ligands 1-6 against CKit2

Circular Dichroism (CD) Studies

The Quadruplex forming human telomeric DNA sequence $d(G_3[TTAG_3]_3$ was purchased from AtdBio Limited, School of Chemistry, University of Southampton. CD spectra were measured on a Jasco J-720 spectropolarimeter. All DNA samples were dissolved in Tris-HCl buffer (50 mM, pH 7.4); the samples also contained 100 mM KCl. The quadruplex sequences were annealed by heating to 90°C and followed by slowly cooling to 15°C over a period of 24 h. 1 mM stock solutions of the ligands in mili Q water were prepared from a 50 mM stock solution in DMSO. Spectra were recorded between 220 - 320 nm and 220 - 400 nm in 5 mm path length cuvettes. These were averaged over 5 scans, which were recorded at 100 nm min^{-1} with a response time of 1 s and a bandwidth of 1 nm. A buffer baseline was subtracted from each spectrum, and the spectra were normalized to have zero ellipticity at 320 nm. The ligands do not have any chiral centre and were CD inactive.

Circular Dichroism (CD) and Quadruplex Topology

Intramolecular quadruplexes can adopt a variety of different topologies in which the strands run in different orientations with lateral, edgewise or diagonal loops. Circular dichroism (CD) can be used to indicate whether these fold in a parallel or antiparallel configuration. Parallel quadruplexes, in which the glycosidic bonds are all *anti*, display a positive CD signal around 265 nm, with a negative peak around 240 nm. In contrast, antiparallel structures, with both *syn* and *anti* bonds, exhibit a positive signal at around 295 nm, with a negative signal or shoulder around 260 nm.

CD Titration with H-Telo G-Quadruplex DNA (in presence of 100 mM KCl):

Quadruplex forming sequence : $d(G_3[TTAG_3]_3$

The CD spectrum of the HT4 sequence showed the presence of mixed parallel and antiparallel structures with positive peaks around 295 nm and a negative peak around 240 nm. In general, titrations with the ligands **1-6** showed selective induction of anti-parallel G-quadruplex structure. For ligand **1-6**, we observed a concentration-dependent enhancement of the major positive peak at 295 nm, concentration dependent appearance of a major negative peak around 260 nm, and concentration dependent disappearance of the negative peak around 240 nm (**Figure S4A-S4E**). These observations are supportive of selective induction of an anti-parallel structure upon addition of the ligands to the native quadruplex sequence.

However, except for **3** and **4**, the ligands showed a reduction in intensity of the CD signal at 295 nm at higher ligand concentrations (higher than 3 equivalents), but all ligands showed concentration dependent appearance of the major negative peak at 260 nm (characteristic of anti-parallel structure) and disappearance of the negative peak at 240 nm (characteristic of parallel structure) at the concentrations used in the study (1-6 equivalents).

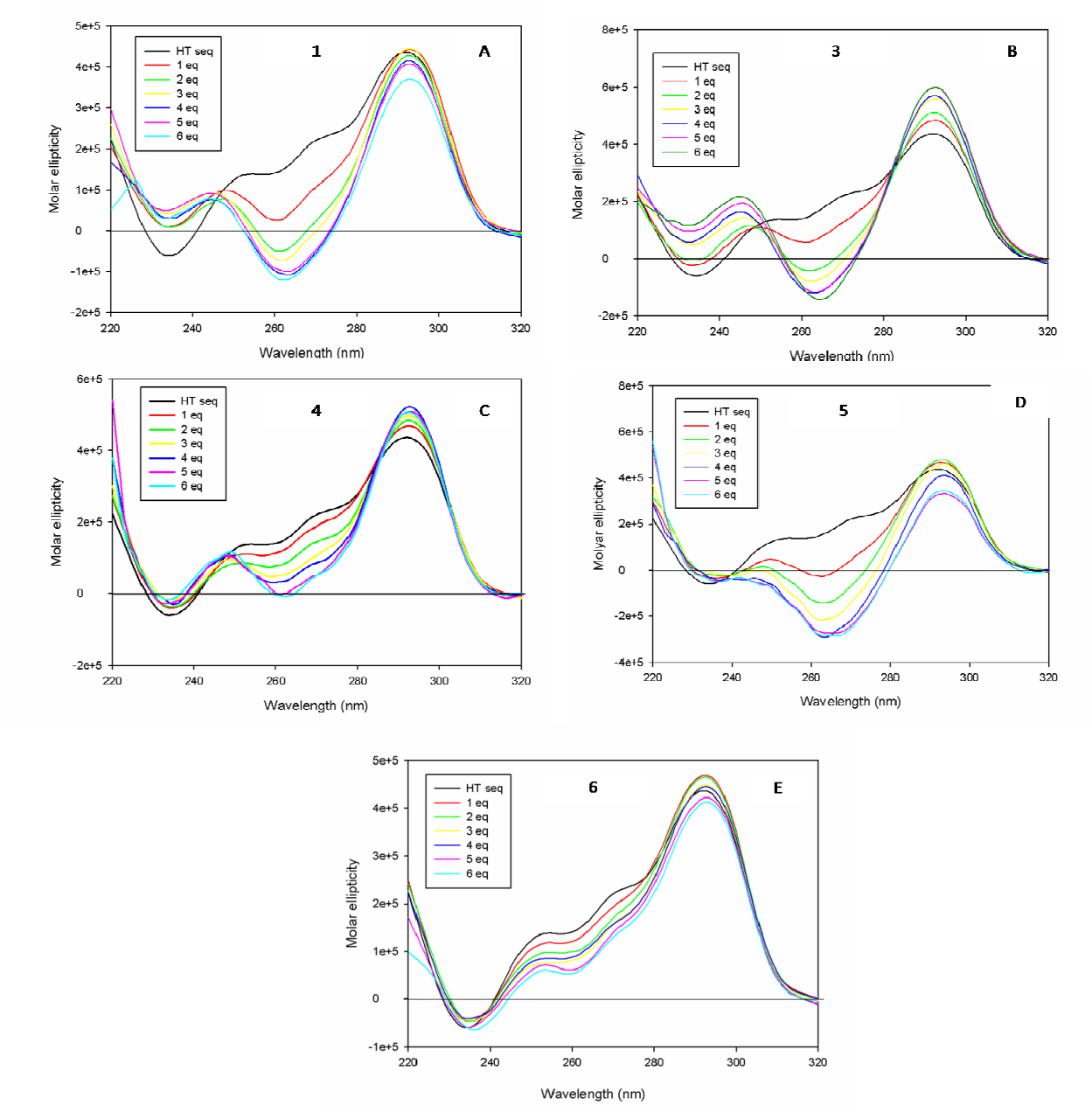


Figure S4: CD spectra of HT4 (*h-telo*): 5 μM in Tris buffer (pH 7.4) in presence of 100 mM KCl, 0-6 equivalent of ligands; A, **1**; B, **3**; C, **4**; D, **5**; E, **6**.

Cell Culture Experiments

All cell lines were purchased from ATCC-LGC Promochem and viability was maintained in a Heraeus Hera Cell 240 incubator (37ºC, 5% CO_2; 75 cm^2 plates supplied by TPP).

The following media were prepared under sterile conditions as follows:
Dulbecco's Modified Eagles Media (DMEM; Invitrogen) supplemented with foetal bovine serum (10% v/v; Invitrogen), hydrocortisone (0.5μg/ml; Acros Organics), L-glutamine (2mM; Invitrogen) and non-essential amino acids (1x; Invitrogen) was used for the sub culturing of MCF7, A549 and HT29 cell lines, and Minimal Essential Medium (MEM; Sigma-Aldrich) supplemented with foetal bovine serum (10% v/v; Invitrogen), L-glutamine (2mM; Invitrogen) and non-essential amino acids (1x; Invitrogen) was used for maintenance of the WI38 cell line.

Sulforhodamine B (SRB) Short Term Cytotoxicity Assay:

Short term growth inhibition was measured using the SRB assay as described previously **(Ref)**. Briefly, cells were seeded (4000 cells/well MCF7 and WI38; 1000 cells/well A549; 200cells/well HT29) into the wells of 96 well-plates in appropriate medi and incubated overnight to allow the cells to attach.

Subsequently, cells were exposed to freshly-made solutions of drug at increasing concentrations between 0.1 – 50 μM in quadruplicate, and incubated for a further 96h. Following this, the cells were fixed with ice cold trichloroacetic acid (TCA) (10% w/v) for 30 min and stained with 0.4% SRB dissolved in 1% acetic acid for 15 mins. All incubations were carried out at room temperature. The IC_{50} values (concentration required to inhibit cell growth by 50%) were determined from the mean absorbance at 540nm for each drug concentration expressed as a percentage of the well absorbance in untreated control cells.

Ref:

M. Gunaratnam, O. Greciano, C. Martins, A. P. Reszka, C. M. Schultes, H. Morjani, J. F. Riou, and S. Neidle, *Biochem. Pharmacol.*, 2007**, 74**, 679.

DNAse I Footprinting Experiments

The HexA sequence was cut from the plasmid using HindIII and SacI and was labelled at the 3'-end of the HindIII site. 1.5 µl radiolabelled DNA was mixed with 1.5 µl ligand solution (dissolved in 10 mM Tris–HCl, pH 7.5 containing 10 mM NaCl). After equilibration of the ligand–DNA complexes the mixtures were digested by adding 2 µl DNase I (about 0.01 U/ml), diluted in 20 mM NaCl, 2 mM $MgCl_2$, 2 mM $MnCl_2$. DNase I was purchased from Sigma and was stored at -20 ºC as a stock concentration of 7200 U/ml in 0.15 M NaCl containing 1 mM $MgCl_2$.

After 1 min the reaction was stopped by adding 4 µl DNase I stop solution (10 mM EDTA, 1 mM NaOH, 0.1% bromophenol blue, 80% formamide). Before loading onto the gel, the DNA was denatured by incubating at 100 ºC for 3 mins followed by rapid cooling on ice before running on an 8% denaturing polyacrylamide gel [16 ml Sequagel (National Diagnostics), 5 ml 10 x TBE Buffer containing 8 M urea, 27 ml diluent (50% urea)]. Gels (40 cm long, 0.3 mm thick) were run at 1500 V for about 2 h until the dye reached the bottom of the gel. The gel plates were then separated and the gel fixed by immersing in 10% (v/v) acetic acid before transferring to Whatman 3MM paper and drying under vacuum at 80 ºC. The dried gel was then exposed to a phosphorimager screen overnight before scanning.

HexA Sequence (Top Strand)

5’-GGATCCCGGGATATCGATATATGGCGCCAAATTTAGCTATAGATCTAGAATTCCGGACCGCGGTTTAAACG
3’-CCTAGGGCCCTATAGCTATATACCGCGGTTTAAATCGATATCTAGATCTTAAGGCCTGGCGCCAAATTTGC
TTAACCGGTACCTAGGCCTGCAGCTGCGCATGCTAGCGCTTAAGTACTAGTGCACGTGGCCATGGATCC-3’
AATTGGCCATGGATCCGGACGTCGACGCGTACGATCGCGAATTCATGATCACGTGCACCGGTACCTAGG-5’

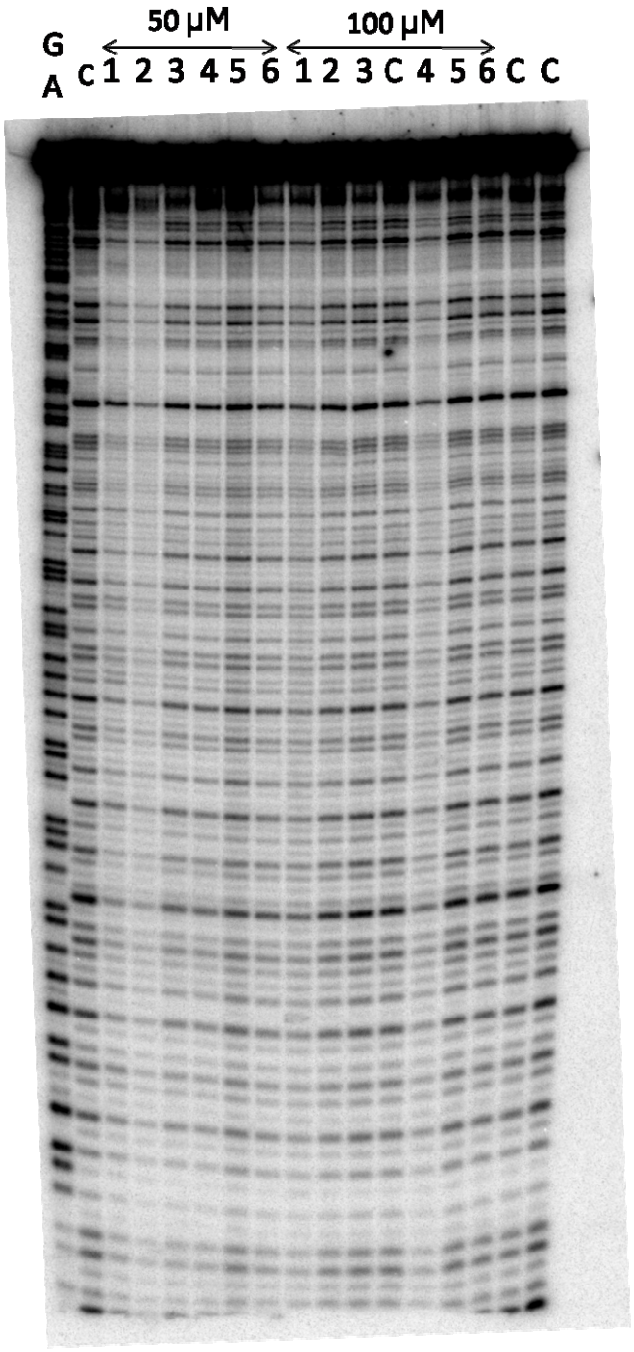


Figure S5: Footprinting gel of ligands **1**-**6** for the Hex A sequence. GA is a marker lane specific for purines; C is the control lane in the absence of any ligand. No DNA binding was observed for any ligand up to a concentration of 100 µM.

Molecular Modelling

Telomeric G-quadruplex can exist in a number of polymorphic forms and using any one of these as a starting point for modelling can be problematic in the absence of direct structural information. In view of these issues, we have used a G-quartet from one of the quadruplex-ligand complex crystal structures as a starting-point. The modelling mainly looked at the stacking of these new class of ligands on the G-quartet. A terminal G-quartet from the crystal structure of a human intramolecular telomeric G-quadruplex complexed with a naphthalene diimide ligand (PDB id 3CDM) was used as a starting-point to study plausible interactions with biaryl polyamides. Automated docking studies were carried out using the AutoDock program. v4.0 (D. S. Goodsell, G. M. Morris and A. J. Olson. *J. Mol. Recogn*., 1996, **9**, 1). A total of 250 independent docking runs were undertaken to enhance the reliability of the docking process (R. Wang, Y. Lu and S. Wang. *J. Med. Chem*., 2003, **46**, 2287). Cluster analysis was carried out on the docked results using a root mean square (RMS) tolerance of 1.0 Å. Figure 1B in the manuscript shows the ligand G-quartet overlap for the lowest-energy solution.