Controlled-Folding of a Small Molecule Modulates DNA G-Quadruplex Recognition

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SUPPORTING INFORMATION for a Communication to Chemical Communications

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1. General Experimental and synthesis

All solvents and reagents were purified by standard techniques reported in Perrin, D. D.; Armarego, W. L. F., Purification of Laboratory Chemicals, 3rd edition, Pergamon Press, Oxford, 1988 or used as supplied from commercial sources, as appropriate. NMR spectra were acquired on Bruker DRX-400, Bruker DPX-400 and DRX-500 instruments using deuterated solvents as detailed and at ambient probe temperature (300 K). Notation for the $^1$H NMR spectral splitting patterns includes: singlet ($s$), doublet ($d$), triplet ($t$), broad ($br$) and multiplet/overlapping peaks ($m$). Signals are quoted as δ values in ppm, coupling constants ($J$), are quoted in Hertz. Mass spectra were recorded on Micromass Q-Tof (ESI) spectrometer. TLC were performed on Merck Kieselgel 60 F254 plates, and spots were visualised under UV light. Flash chromatography (FC) were performed using Merck Kieselgel 60 at RT under a positive pressure of nitrogen using previously distilled solvents.

HPLC purification was done by using a Varian Pursuit C18, 5 µ column (250 × 21.2 mm) and a gradient elution with 0.1% TFA/ H2O (solvent A) and 0.1% TFA/ MeCN (solvent B) at a flow rate of 12.0 ml/ min.

All DNA oligonucleotides were synthesised and supplied by Eurogentec® Ltd.
4-(2-Pyrrolidinyl-ethoxy)-quinolin-2-ylamine (6b)

2-Amino-quinolinone (1.5 g, 9.4 mmol), N-(2-hydroxyethyl)-pyrrolidine (1.7 g, 14.5 mmol) and triphenylphosphine (4.9 g, 18.6 mmol) were dissolved in 100 ml of freshly distilled THF and cooled to 0°C. DIAD (3.8 g, 18.6 mmol) was added dropwise under argon. The mixture was allowed to warm to rt and stirred for 3 d. The solvent was removed \textit{in vacuo} and the product purified by column chromatography (87% EtOAc, 10% MeOH, 3% TEA) to obtain the title compound as a light yellow powder (1.3 g, 5.0 mmol, 53% yield). \textit{^1}{H NMR (400 MHz, CD$_3$OD)} $\delta_{H}$ 8.00 (1H, dd, $J$ 8.0, 1.0 Hz), 7.53 (1H, dd, $J$ 8.0, 1.5 Hz), 7.48 (1H, ddd, $J$ 8.0, 6.5, 1.0 Hz), 7.20 (1H, ddd, $J$ 8.0, 6.5, 1.5 Hz), 6.28 (1H, s), 4.33 (2H, $t$, $J$ 5.5 Hz), 3.10 (2H, $t$, $J$ 5.5 Hz), 2.81-2.73 (4H, $m$), 1.94-1.82 (4H, $m$); \textit{^13}{C NMR (100 MHz, CD$_3$OD)} $\delta_{C}$ 163.0, 160.3, 148.0, 130.2, 124.1, 121.9,
121.6, 110.8, 90.4, 67.6, 54.8, 54.6, 23.3; **HRMS (ES)** calculated for C_{15}H_{20}N_{3}O ([M + H]^+) m/z: 258.1606, found 258.1611.

**4-(2-tert-Butoxycarbonylamino-ethoxy)-quinolin-2-ylamine (6c)**

2-Amino-quinolinone (1.0 g, 6.2 mmol), N-Boc-ethanolamine (1.5 g, 9.3 mmol) and triphenylphosphine (3.3 g, 12.6 mmol) were dissolved in 100 ml of freshly distilled THF and cooled to 0 °C. DIAD (1.8 ml, 9.4 mmol) was added dropwise under argon. The mixture was allowed to warm to rt and stirred for 3 d. The solvent was removed in vacuo and the product purified by column chromatography (90% EtOAc, 10% MeOH) to obtain the title compound as a white powder (1.2 g, 4.0 mmol, 65% yield). **1H NMR (500 MHz, CDCl₃)** δH 7.97 (1H, dd, J 8.0, 1.0 Hz), 7.59 (1H, dd, J 8.5, 1.0 Hz), 7.54 (1H, ddd, J 8.5, 7.0, 1.0 Hz), 7.23 (1H, ddd, J 8.0, 7.0, 1.0 Hz), 6.02 (1H, s), 5.00 (1H, br s), 4.69 (2H, br s), 4.16 (2H, t, J 5.0 Hz), 3.67 (2H, dd, J 5.0, 5.5), 1.47 (9H, s); **13C NMR (125 MHz, CDCl₃)** δC 162.3, 158.0, 155.9, 148.5, 130.3, 125.7, 122.0, 121.6, 117.5, 90.1, 79.8, 67.5, 39.8, 28.4; **HRMS (ES)** calculated for C_{16}H_{22}N_{3}O_{3} ([M + H]^+) m/z: 304.1650, found 304.1668.

**General procedure for compounds 1a-3b**

Two equivalents of 6c (for 1a, 2a and 3a) or 6b (for 1b, 2b and 3b) were dissolved in 2 ml of dry chloroform and one equivalent of 1,3- diisocyanato benzene (for 1a and 1b), 2,4-toluene diisocyanate (for 2a and 2b) or 2,6-toluene diisocyanate (for 3a and 3b) was added. The mixture was heated to 50°C and allowed to stir overnight. The solvent was removed in vacuo and the mixture redissolved in 3 ml DCM and 1 ml TFA. The mixture was allowed to stir for 1 h and then the solvent removed in vacuo to yield a light yellow solid. Compounds were purified by HPLC (gradient: 10 to 100% MeCN, 0.1% TFA over 20 min, Rₑ=13.0-16.0 min) to yield the TFA salts of the products as white powders.
1,3-[di(4-{2-Amino-ethoxy}-quinolin-2-yl)-diureido] benzene (1a)

Reacting 6c (133 mg, 0.44 mmol) with 1,3-phenylene diisocyanate (35 mg, 0.22 mmol) afforded the title compound (154 mg, 0.19 mmol, 86% yield). $^1$H NMR (500MHz, D$_2$O) $\delta$H 8.31 (2H, d, J 8.0 Hz), 7.78 (2H, d, J 8.0 Hz), 7.69 (2H, t, J 8.0 Hz), 7.60 (1H, s), 7.48 (2H, t, J 8.0 Hz), 7.35 (2H, d, J 8.0 Hz), 7.20 (1H, t, J 8.0 Hz), 6.69 (2H, s), 4.59 (4H, t, J 5.0 Hz), 3.61 (4H, t, J 5.0 Hz); $^{13}$C NMR (125MHz, D$_2$O) $\delta$C 162.5, 163.0, 154.1, 153.9, 139.8, 134.2, 130.7, 127.5, 127.4, 124.1, 118.9, 116.1, 111.0, 93.1, 68.0, 39.7; HRMS (ES) calculated for C$_{30}$H$_{31}$N$_8$O$_4$ ([M + H]$^+$) m/z: 567.2463, found 567.2456.

2,4-[di(4-{2-Amino-ethoxy}-quinolin-2-yl)-diureido] toluene (2a)

Reacting 6c (118 mg, 0.40 mmol) with 2,4-toluene diisocyanate (34 mg, 0.20 mmol) afforded the title compound (150 mg, 0.18 mmol, 90% yield). $^1$H NMR (500MHz, D$_2$O) $\delta$H 8.28 (1H, d, J 7.5 Hz), 8.24 (1H, d, J 7.5 Hz), 8.20 (1H, s), 7.75-7.70 (2H, m), 7.70-7.62 (2H, m), 7.48 (1H, t, J 7.5 Hz), 7.43 (1H, t, J 7.5 Hz), 7.11 (1H, d, J 8.5 Hz), 6.98 (1H, d, J 8.5 Hz), 6.73 (2H, br s), 4.60-4.56 (2H, m), 4.56-4.50 (2H, m), 3.61-3.53 (4H, m), 2.19 (3H, s); $^{13}$C NMR (125MHz, D$_2$O) $\delta$C 163.2, 162.9, 162.6, 162.3, 154.3, 153.8, 153.9, 137.3, 134.5, 134.0, 131.8, 127.4, 126.9, 126.8, 124.3, 124.0, 122.0, 119.6, 119.5, 118.9, 118.7, 117.2, 116.6, 114.0, 93.3, 93.1, 67.9, 67.6, 39.7, 39.6, 18.2; HRMS (ES) calculated for C$_{31}$H$_{33}$N$_8$O$_4$ ([M + H]$^+$) m/z: 581.2619, found 581.2637.

2,6-[di(4-{2-Amino-ethoxy}-quinolin-2-yl)-diureido] toluene (3a)

Reacting 6c (97 mg, 0.32 mmol) with 2,6-toluene diisocyanate (28 mg, 0.16 mmol) afforded the title compound (117 mg, 0.14 mmol, 88% yield). $^1$H NMR (500MHz, D$_2$O) $\delta$H 8.26 (2H, d, J 8.0 Hz), 7.88 (2H, d, J 8.0 Hz), 7.84 (2H, t, J 8.0 Hz), 7.64 (2H, t, J 8.0 Hz), 7.41 (2H, d, J 8.5 Hz), 7.35 (1H, d, J 8.5 Hz), 6.74 (2H, s), 4.65 (4H, t, J 5.0 Hz), 3.62 (4H, t, J 5.0 Hz), 2.40 (3H, s); $^{13}$C NMR (125MHz, D$_2$O) $\delta$C 162.1, 161.8, 154.9, 154.2, 137.1, 134.7, 127.6, 127.4, 124.4, 123.3, 119.0, 118.8, 116.5, 93.3, 68.0, 39.6, 13.3; HRMS (ES) calculated for C$_{31}$H$_{33}$N$_8$O$_4$ ([M + H]$^+$) m/z: 581.2619, found 581.2637.
1,3-[di(4-{2-Pyrrolidinyl-ethoxy}-quinolin-2-yl) diureido] benzene (1b)

Reacting 6b (56 mg, 0.22 mmol) with 1,3 phenylene diisocyanate (18 mg, 0.11 mmol) afforded the title compound (90 mg, 0.10 mmol, 91% yield).

\[
\begin{align*}
\text{\^{1}H NMR (500MHz, DMSO-d6)} & \delta_H 12.00 (2H, \text{ br s}), 10.12 (2H, \text{ br s}), 8.23 (2H, dd, J 8.0, 1.0 Hz), 8.05-8.02 (1H, m), 7.90 (2H, dd, J 8.5, 1.0 Hz), 7.77 (2H, ddd, J 8.5, 7.0, 1.0 Hz), 7.49 (2H, ddd, J 8.0, 7.0, 1.0), 7.41-7.37 (2H, m), 7.36-7.32 (1H, m), 6.96 (2H, s), 4.55 (4H, t, J 4.0), 3.81 (4H, t, J 4.0), 3.76-3.66 (4H, m), 3.31-3.19 (4H, m), 2.14-2.03 (4H, m), 1.97-1.86 (4H, m);
\end{align*}
\]

\[
\text{\^{13}C NMR (125MHz, DMSO-d6)} \delta_C 161.8, 153.6, 152.3, 145.2, 139.5, 131.2, 129.7, 125.6, 124.3, 122.3, 117.9, 117.4, 115.1, 112.8; \text{HRMS (ES) calculated for C}_{38}\text{H}_{43}\text{N}_{8}\text{O}_{4} ([M + H]^+) m/z: 675.3407, found 675.3411.
\]

2,4-[di(4-{2-Pyrrolidinyl-ethoxy}-quinolin-2-yl) diureido] toluene (2b)

Reacting 6b (49 mg, 0.20 mmol) with 2,4-toluene diisocyanate (17 mg, 0.10 mmol) afforded the title compound (85 mg, 0.09 mmol, 90% yield).

\[
\begin{align*}
\text{\^{1}H NMR (500MHz, DMSO-d6)} & \delta_H 11.80 (2H, \text{ br s}), 10.10 (2H, \text{ br s}), 8.44-8.40 (1H, m), 8.22 (2H, br d, J 9.0 Hz), 7.81 (1H, d, J 8.0 Hz), 7.84 (1H, d, J 8.0), 7.76 (2H, t, J 8.0 Hz), 7.51-7.43 (3H, m), 7.27-7.23 (1H, m), 7.00 (1H, s), 6.82 (1H, s), 4.60-4.50 (4H, m), 3.85-3.78 (4H, m), 3.75-3.66 (4H, m), 3.30-3.20 (4H, m), 2.57 (3H, s), 2.13-2.04 (4H, m), 1.97-1.87 (4H, m); \text{\^{13}C NMR (125MHz, DMSO-d6)} \delta_C 161.8, 161.5, 153.8, 153.6, 152.5, 152.2, 146.0, 137.7, 137.1, 131.2, 131.1, 130.6, 130.5, 125.6, 125.5, 124.2, 124.1, 122.4, 122.3, 121.4, 119.8, 117.9, 117.8, 117.5, 115.1, 113.7, 111.4, 92.8, 92.7, 64.4, 64.3, 54.2, 52.8, 22.7, 18.3; \text{HRMS (ES) calculated for C}_{39}\text{H}_{45}\text{N}_{8}\text{O}_{4} ([M + H]^+) m/z: 689.3564, found 689.3569.
\end{align*}
\]

2,6-[di(4-{2-Pyrrolidinyl-ethoxy}-quinolin-2-yl) diureido] toluene (3b)

Reacting 6b (62 mg, 0.22 mmol) with 2,6-toluene diisocyanate (21 mg, 0.12 mmol) afforded the title compound (103 mg, 0.11 mmol, 92% yield);

\[
\begin{align*}
\text{\^{1}H NMR (500MHz, DMSO-d6)} & \delta_H 11.70 (2H, \text{ br s}), 10.25 (2H, \text{ br s}), 8.23 (2H, d, J 8.0 Hz), 7.88 (2H, d, J 8.0 Hz), 7.79 (2H, d, J 8.0 Hz), 7.76 (2H, t, J 8.0 Hz), 7.48 (2H, t, J 8.0 Hz), 7.24 (1H, t, J 8.0 Hz), 6.89 (2H, s), 4.55 (4H, m), 3.87-3.79 (4H, m), 3.76-3.67 (4H, m), 3.30-3.20 (4H, m), 3.56 (3H, s), 2.13-2.04 (4H, m), 1.96-1.88 (4H, m); \text{\^{13}C NMR}
\end{align*}
\]
Supplementary Material (ESI) for Chemical Communications 
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(125MHz, DMSO-d<sup>6</sup>) δ<sub>C</sub> 161.8, 153.8, 152.6, 145.1, 137.4, 131.2, 126.0, 125.2, 124.2, 122.4, 119.7, 117.9, 117.2, 114.8, 112.5, 92.7, 64.4, 54.1, 52.7, 22.7, 13.6; HRMS (ES) calculated for C<sub>39</sub>H<sub>45</sub>N<sub>8</sub>O<sub>4</sub> ([M + H]<sup>+</sup>) m/z: 689.3564, found 689.3574.
2. 2D NMR and 1D NOeSY spectra of compounds 2a and 3a

The COSY spectrum was used to assign the protons and investigate special interactions in the NOESY spectrum.

2a

![Diagram of compound 2a]

The numbers donate the types of protons on the respective carbons.

3a

![Diagram of compound 3a]

The numbers donate the types of protons on the respective carbons.
It can be seen that the hydrogens on the methyl group (4) couple with the aromatic hydrogen 6a via nOe. They must be in close special proximity and hence one of the quinolines must be folded up.
It can be seen that the hydrogens on the methyl group (4) couple with the aromatic hydrogens 5 via nOe. They must be in close vicinity and hence one both quinolines must be folded down.
We also performed 1D NOESY experiments in CD$_3$OD by radiating the samples using the frequency of the methyl hydrogens (3) to confirm that the cross peaks observed were due to magnetization transfer by Nuclear Overhauser effect. Data for 3a shown below.
3. **FRET melting experiments**

Oligonucleotides were initially dissolved as a 100 μM stock solution in MilliQ water; further dilutions were carried out in 60 mM potassium cacodylate buffer, pH 7.4 and FRET experiments were carried out with a 200 nM oligonucleotide solution. Six DNA oligonucleotides were used in these experiments: which were dual fluorescently labeled.

**K-ras**\(^1\) was a dual-labeled 32-mer oligonucleotide comprising a quadruplex forming region in the promoter region of the human K-ras gene, 5′-FAM-AGG GCG GTG GAA GAG GGA AGA GGG GGA GG-TAMRA-3′

**c-kit1** \(^2\) was a dual-labeled 21-mer oligonucleotide comprising one of the quadruplex forming regions in the promoter region of the human c-kit oncogene, 5′-FAM-GGG AGG GCG CTG GGA GGA GGG-TAMRA-3′

**h-Telo** \(^3\) was a dual-labeled 21-mer oligonucleotide comprising the minimum human telomeric G-overhang sequence required to fold into an intramolecular G-quadruplex, 5′-FAM-GGG TTA GGG TTA GGG TTA GGG-TAMRA-3′

**c-kit2** \(^4\) was a dual-labeled 20-mer oligonucleotide comprising one of the quadruplex forming regions in the promoter region of the human c-kit oncogene, 5′-FAM-GGG CGG GCG CGA GGG AGG GG-TAMRA-3′.

**c-myc** \(^5\) was a dual-labeled 22-mer oligonucleotide comprising one of the quadruplex forming regions in the promoter region of the human c-kit oncogene, 5′-FAM-TGA GGG TGG GTA GGG TGG GTA A-TAMRA-3′.

**ds-DNA** was a dual-labeled 20-mer oligonucleotide comprising a self-complementary sequence with a central polyethylene glycol linker able to fold into a hairpin, 5′-FAM-TAT AGC TAT A HEG TAT AGC TAT A-TAMRA-3′.

The donor fluorophore was 6-carboxyfluorescein, FAM, and the acceptor fluorophore was 6-carboxytetramethylrhodamine, TAMRA. Dual-labeled DNA was annealed at a concentration of 400 nM by heating at 94 °C for 10 min followed by

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cooling to rt at a rate of 0.1 °C/min. 96-well plates were prepared by addition of 50 μl of the annealed DNA solution to each well, followed by 50 μl of a solution of the respective molecule at an appropriate concentration. Measurements were made in triplicate with an excitation wavelength of 483 nm and a detection wavelength of 533 nm using a LightCycler® 480 System RT-PCR machine (Roche). Final analysis of the data was carried out using OriginPro 7.5 data analysis and graphing software (OriginLab®).

FRET melting curves for compounds 1b, 2b and 3b: (●) KRAS, (○) c-kit1,(●) h-Telo, (●) c-kit2, (●) c-myc, (●) ds-DNA.
4. HyperChem® modeling

Molecular modeling calculations were performed for molecules 1a, 2a and 3a using HyperChem®, v 8.1 in order to calculate the rotational barriers for C16-C13, C8-C4, C2-C7 and C10-C15 bonds (numbering of the atoms as in the picture below)

Conformational search using the PM3 semi-empirical method with convergence criteria of 0.025 kcal/mol Å was performed for the bonds indicated above (simultaneous rotations). The conformations with the lowest energies were further refined using PM3 with a convergence criteria of 0.01 kcal/mol Å. The optimized structures were placed in a 42875 Å³ “water box” containing 1378 water molecules. The system was optimized using the MM+ force field a convergence criteria 0.025 kcal/mol Å. Pictures of the simulation of the molecules in a water box are displayed on the following pages.
Optimized structure of 1a placed in a 42875 Å³ water box.
Optimized structure of 2a placed in a 42875 Å³ water box.
Optimized structure of 3a placed in a 42875 Å³ water box.