Supplementary Material (ESI) for *Chemical Communications*

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

Sebastian Müller, G. Dan Pantoş, Raphaël Rodriguez and

S. Balasubramanian

The University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK

E-mail: sb10031@cam.ac.uk

SUPPORTING INFORMATION for a Communication to *Chemical Communications*

Contents

1.	**General Experimental and Synthesis**	**S2**
2.	**2D NOESY and 1D nOe spectra**	**S8**
3.	**FRET-melting experiments**	**S12**
4.	**HyperChem® modeling**	**S14**

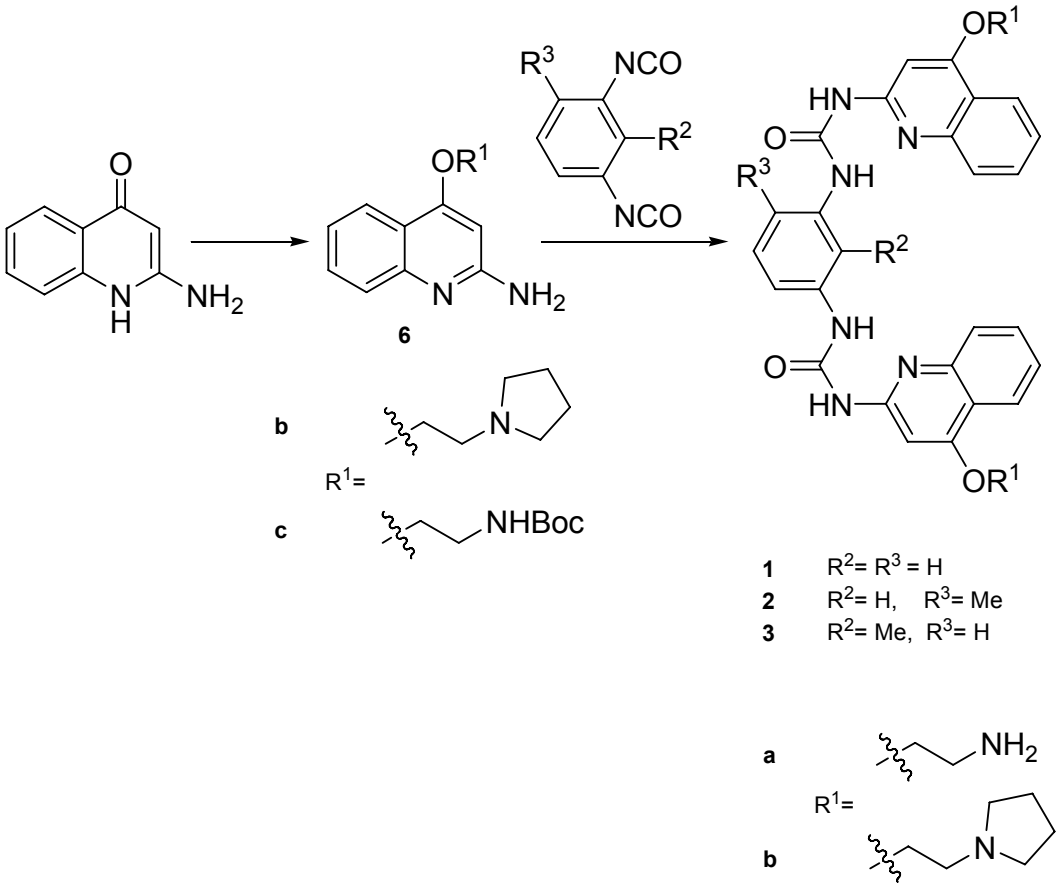
1. General Experimental and synthesis

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/ H_2O (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.

General reaction scheme for ligands 1a-3b



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 *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). **^{1}H NMR (400 MHz, CD_3OD)** δ_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*); **^{13}C NMR (100 MHz, CD_3OD)** δ_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 $C_{15}H_{20}N_3O$ ($[M + H]^+$) 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). **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 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*); **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 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 $C_{16}H_{22}N_3O_3$ ($[M + H]^+$) 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, R_t=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_2O)** δ_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_2O)** δ_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 $C_{30}H_{31}N_8O_4$ ($[M + H]^+$) 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_2O)** δ_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_2O)** δ_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 $C_{31}H_{33}N_8O_4$ ($[M + H]^+$) 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_2O)** δ_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_2O)** δ_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 $C_{31}H_{33}N_8O_4$ ($[M + H]^+$) 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). **^{1}H NMR (500MHz, DMSO-d^{6})** δ_H 12.00 (2H, *br s*), 10.12 (2H, *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*); **^{13}C NMR (125MHz, DMSO-d^{6})** δ_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; **HRMS (ES)** calculated for $C_{38}H_{43}N_8O_4$ ($[M + H]^+$) 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). **^{1}H NMR (500MHz, DMSO-d^{6})** δ_H 11.80 (2H, *br s*), 10.10 (2H, *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*); **^{13}C NMR (125MHz, DMSO-d^{6})** δ_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; **HRMS (ES)** calculated for $C_{39}H_{45}N_8O_4$ ($[M + H]^+$) m/z: 689.3564, found 689.3569.

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

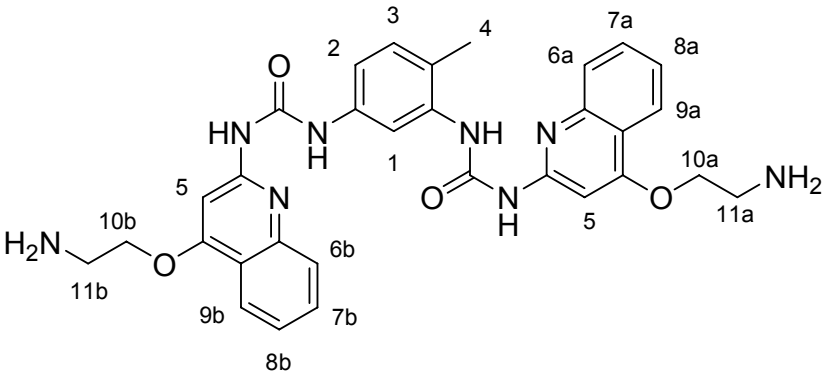
Reacting **6b** (62 mg, 0.22 mmol) with 2,6-toluene diisocyanate (21 mg, 0.12 mmol) afforded the title compound (103 mg, 0.11 mmol, 92% yield); **^{1}H NMR (500MHz, DMSO-d^{6})** δ_H 11.70 (2H, *br s*), 10.25 (2H, *br s*), 8.23 (2H, *d*, *J* 8.0 Hz), 7.88 (2H, *d*, *J* 8.0 Hz), 7.79 (2H, *d*, *J* 8.0 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*); **^{13}C NMR**

(125MHz, DMSO-d^6) δ_C 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 $C_{39}H_{45}N_8O_4$ ($[M + H]^+$) m/z: 689.3564, found 689.3574.

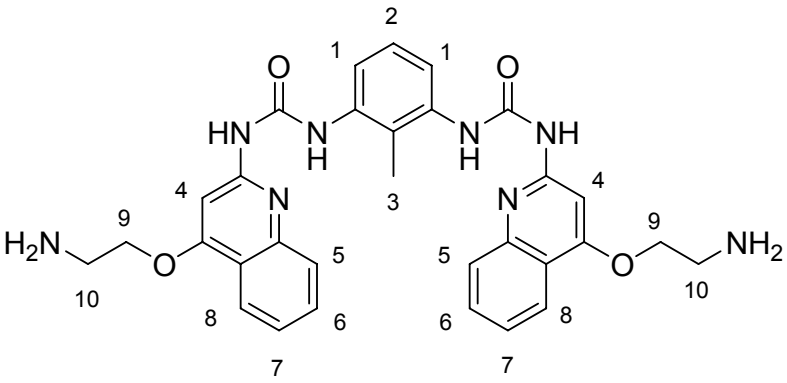
2. 2D NMR and 1D NOeSY spectra of compounds 2a and 3a

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

2a

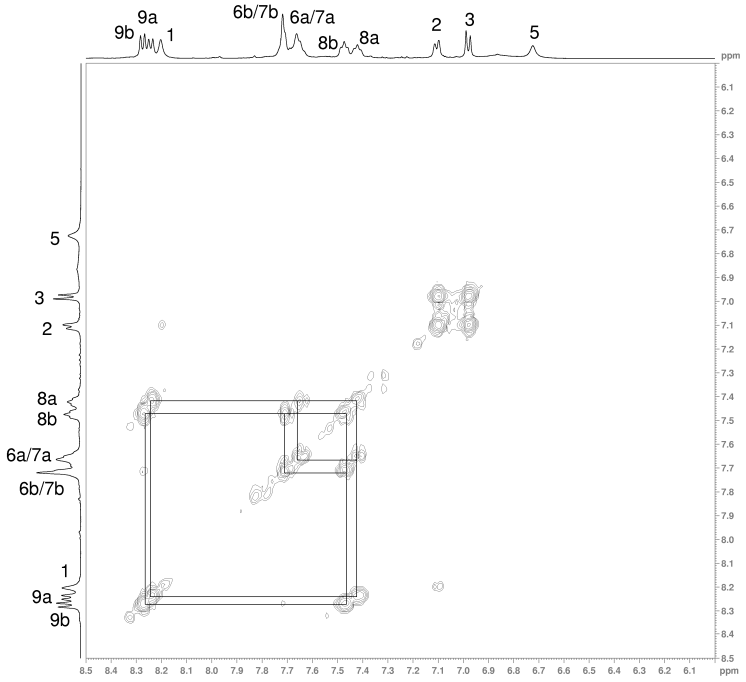


3a

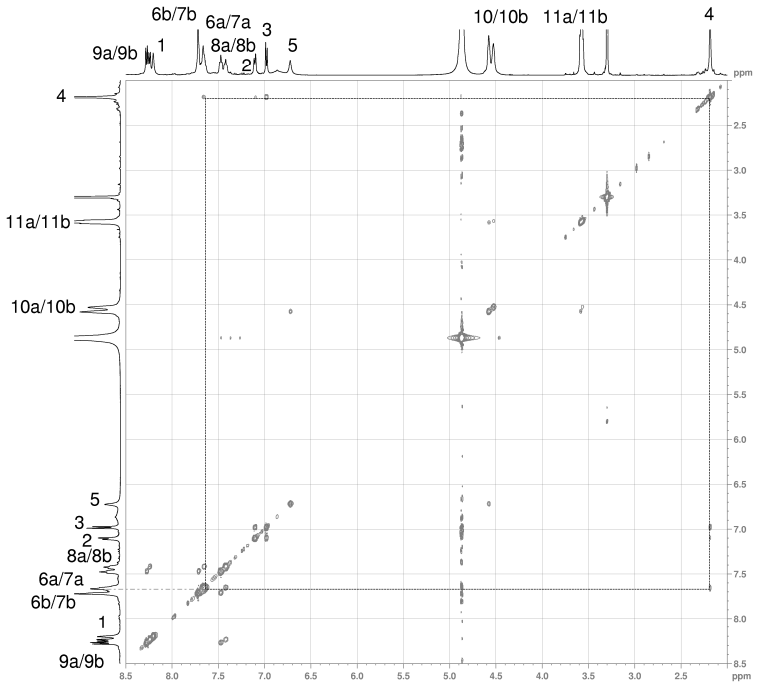


The numbers donate the types of protons on the respective carbons.

COSY for compound **2a** in D_2O

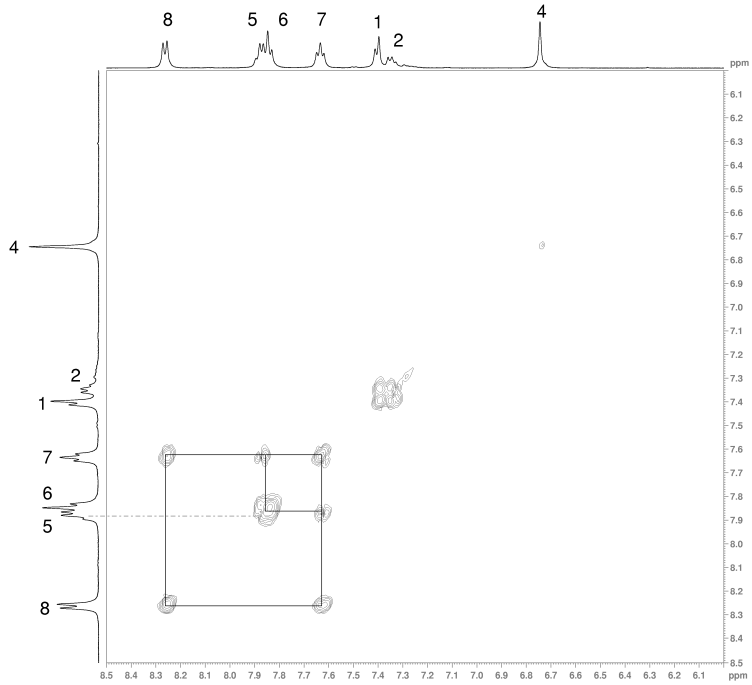


NOESY for compound **2a** in D_2O

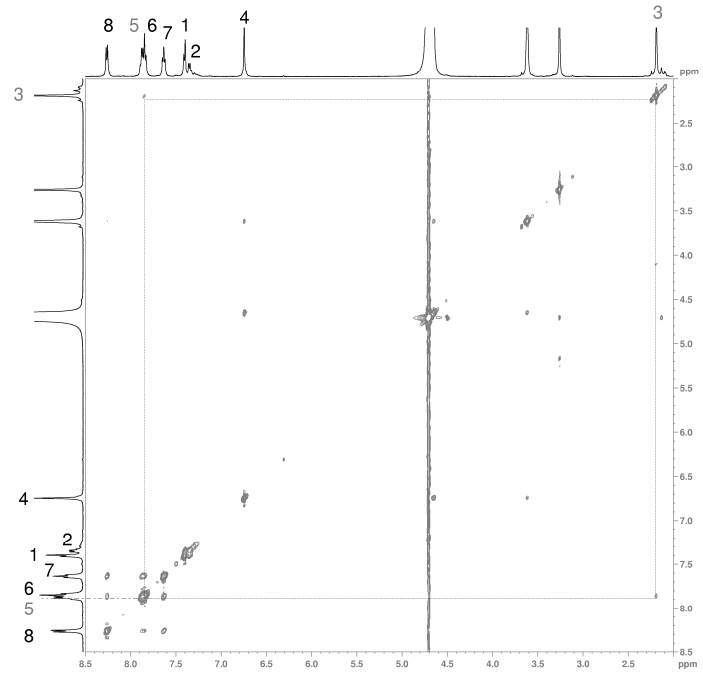


It can be seen that the hydrogens on the methyl group (4) couple with the aromatic hydrogen 6a via nOe. They must be in close special proximity and hence one of the quinolines must be folded up.

COSY for compound **3a** in D_2O

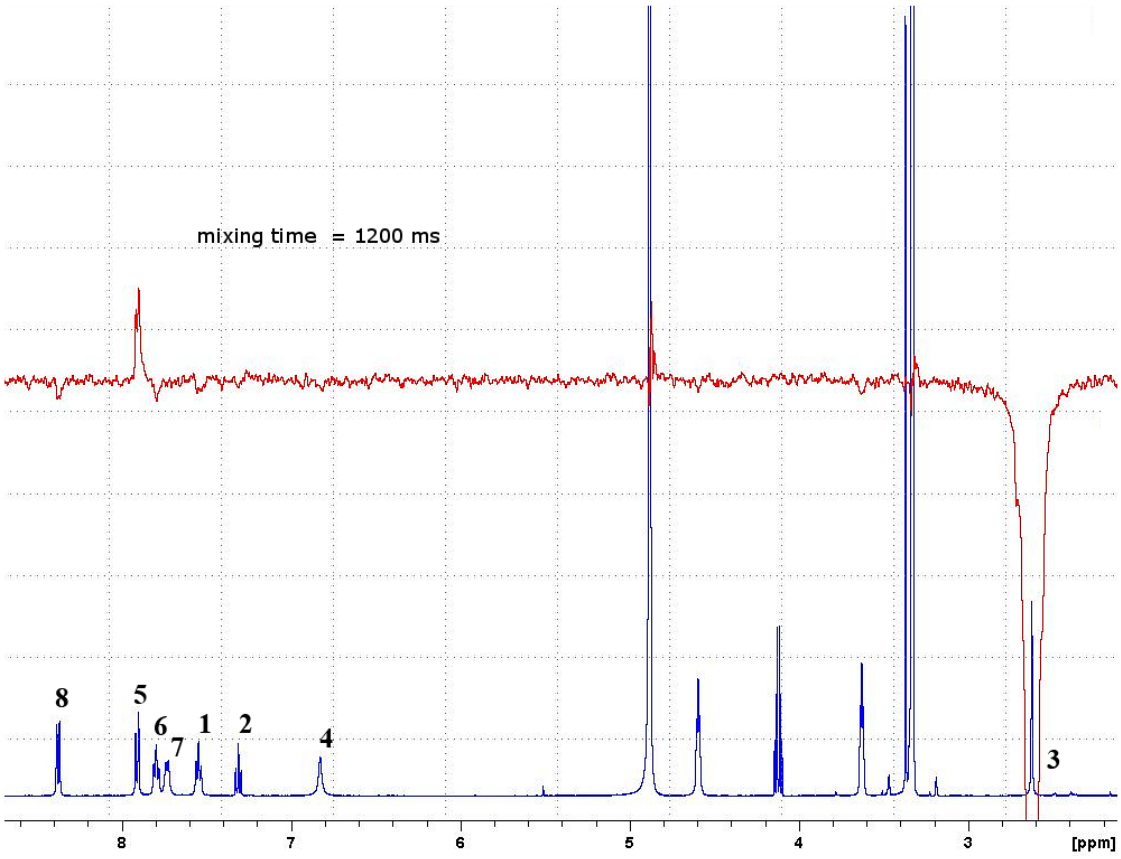


NOESY for compound **3a** in D_2O



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_3OD 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 TGG 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′.

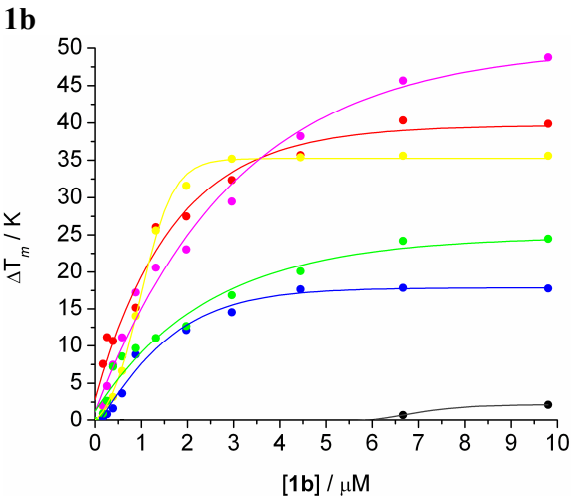
ds-DNA was a dual-labeled 20-*mer* oligonucleotide comprising a self-complementary sequence with a central polyethylene glycol linker able to fold into a hairpin, 5′-FAM-TAT AGC TAT A HEG TAT AGC TAT A-TAMRA-3′.

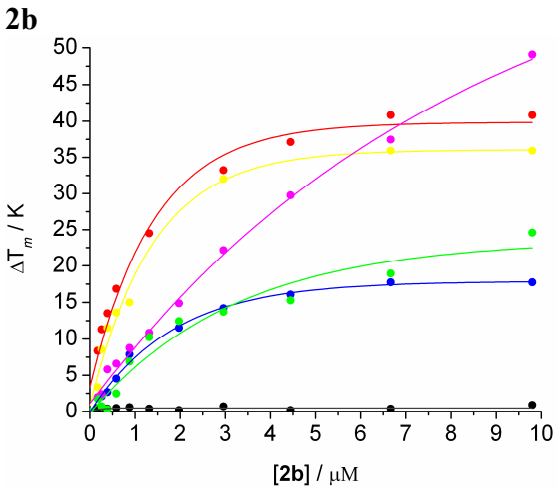
The donor fluorophore was 6-carboxyfluorescein, FAM, and the acceptor fluorophore was 6-carboxytetramethylrhodamine, TAMRA. Dual-labeled DNA was annealed at a concentration of 400 nM by heating at 94 ºC for 10 min followed by

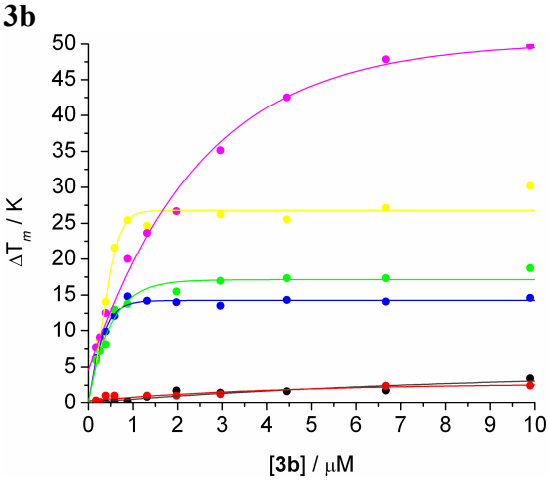
[1] S. Cogoi, M. Paramasivam, B. Spolaore, L. E. Xodo, *Nucleic Acids Res.* 2008, **36**, 3765.
[2] S. Rankin, A. P. Reszka, J. Huppert, M. Zloh, G. N. Parkinson, A. K. Todd, S. Ladame S. Balasubramanian, S. Neidle, *J. Am. Chem. Soc.* 2005, **127**, 10584.
[3] K. Jantos, R. Rodriguez, S. Ladame, P. S. Shirude, S. Balasubramanian, *J. Am. Chem. Soc.* 2006, **128**, 13662.
[4] H. Fernando, A. P. Reszka, J. Huppert, S. Ladame, S. Rankin, A. R. Venkitaraman, S. Neidle, S. Balasubramanian, *Biochemistry* 2006, **45**, 7854.
[5] A. T. Phan, V. Kuryavyi, H. Y. Gaw, D. J. Patel, *Nat. Chem. Biol.* 2005, **1**, 167.

cooling to rt at a rate of 0.1 ºC/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.

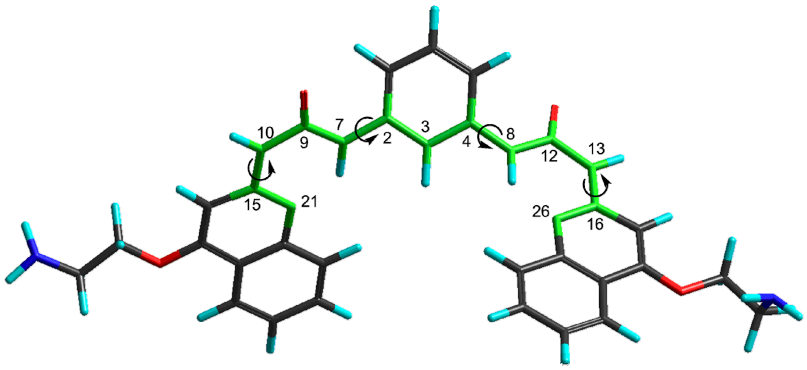




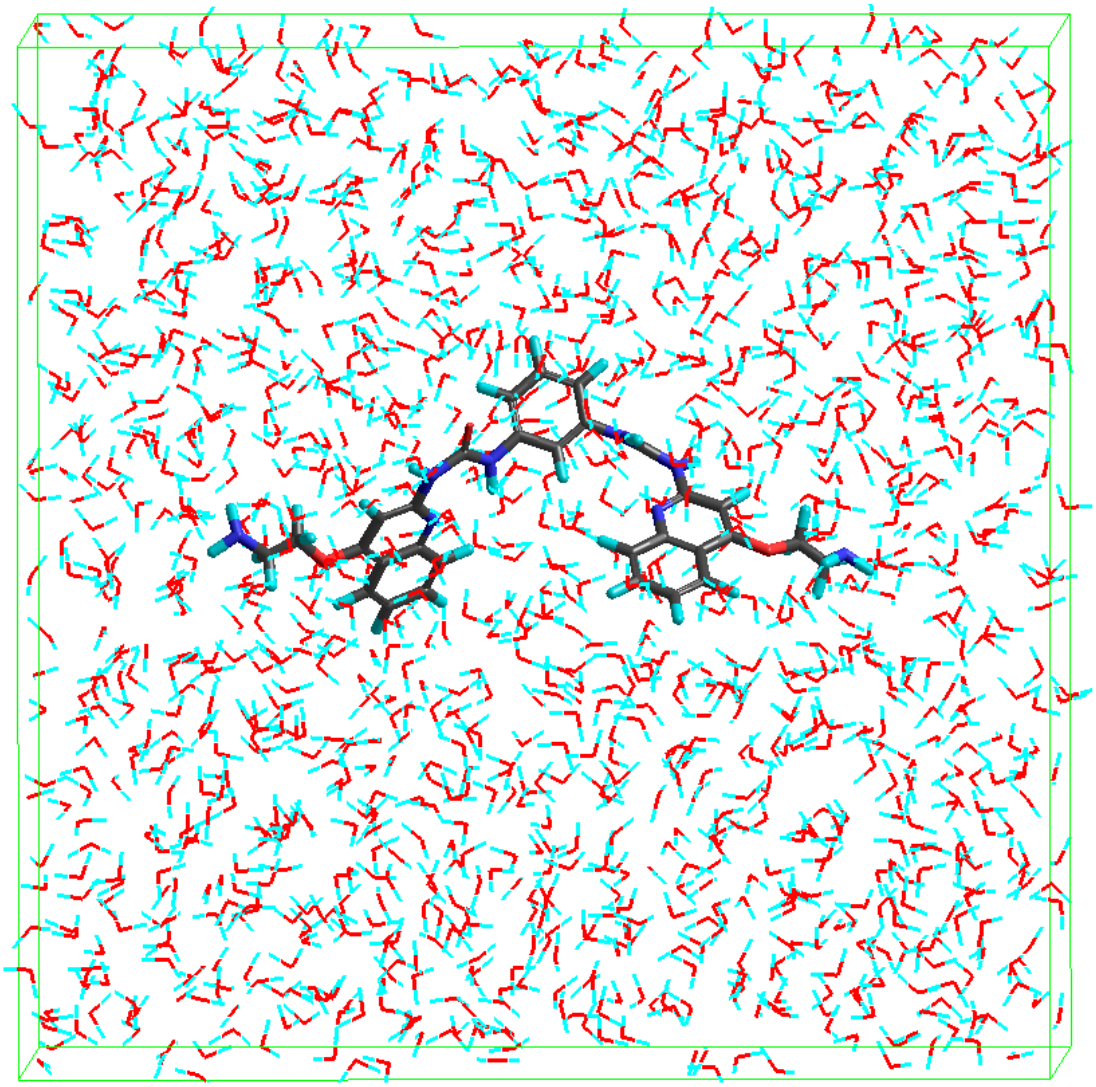


4. HyperChem® modeling

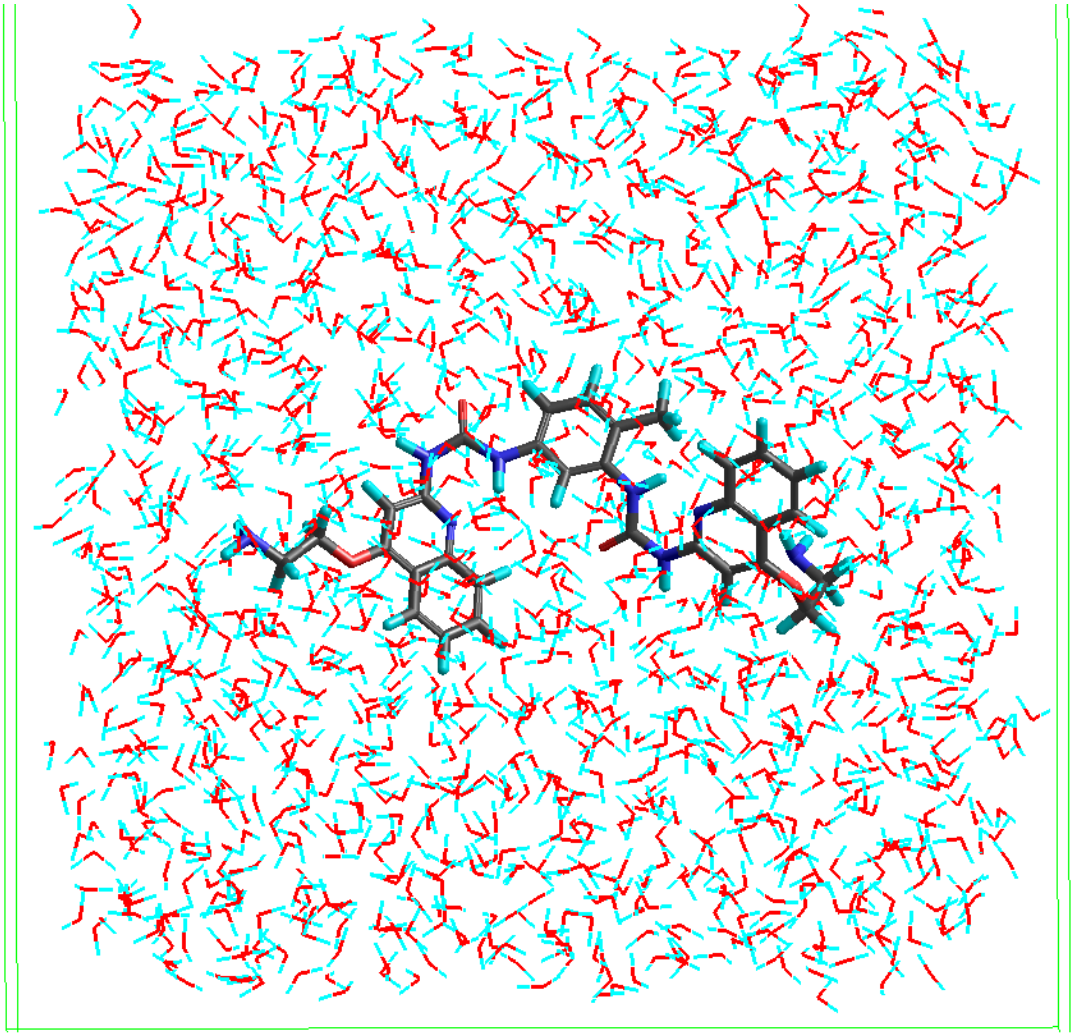
Molecular modeling calculations were performed for molecules **1a**, **2a** and **3a** using HyperChem®, v 8.1 in order to calculate the rotational barriers for C16-C13, C8-C4, C2-C7 and C10-C15 bonds (numbering of the atoms as in the picture below)



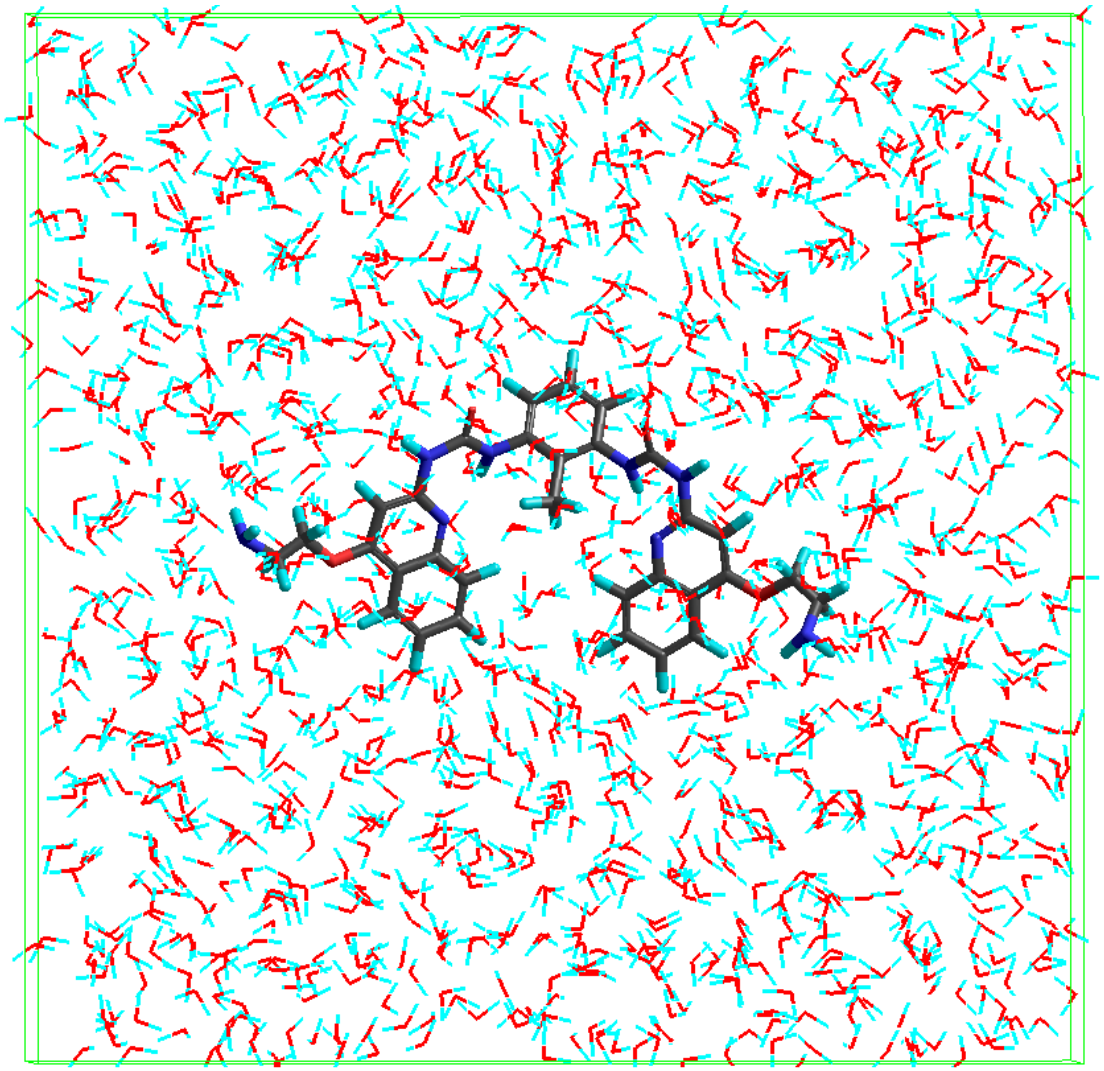
Conformational search using the PM3 semi-empirical method with convergence criteria of 0.025 kcal/ mol Å was performed for the bonds indicated above (simultaneous rotations). The conformations with the lowest energies were further refined using PM3 with a convergence criteria of 0.01 kcal/mol Å. The optimized structures were placed in a 42875 $Å^3$ "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 Å^3 water box.



Optimized structure of **2a** placed in a 42875 $Å^3$ water box.



Optimized structure of **3a** placed in a 42875 Å^3 water box.