Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications


# Supporting Information

# Two-in-one: a pH-sensitive, acridine-based, fluorescent probe binds G-quadruplexes in oncogene promoters.

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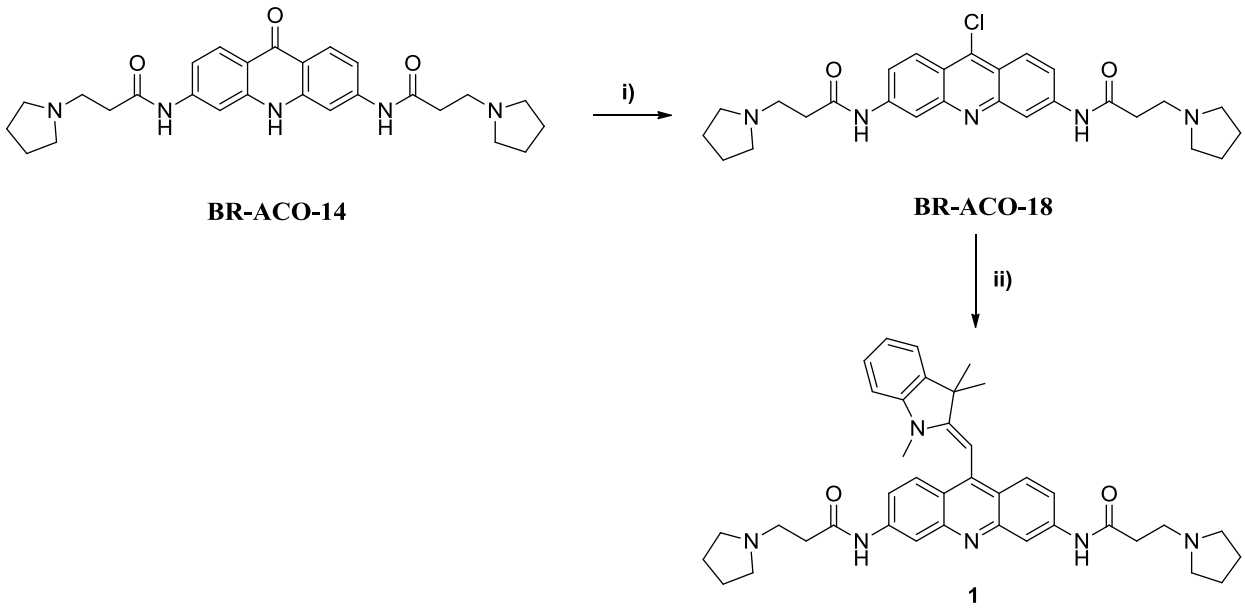
# 1. Experimental Details and Materials




**Scheme S1: i**) $POCl_3$, reflux, 2h, $N_2$. **ii**) 2-methylene-1,3,3-trimethylindoline (50 eq.), $CHCl_3$, 60°C, 6 days, $N_2$. Yield 12%.

All reactions were performed with commercial available reagents and they were used without further purification. $^1H$, $^{13}C$, NMR spectra were recorded on Bruker spectrometer at 400 MHz and at 100 MHz respectively, and the chemical shifts are reported relative to TMS. The structure of **1** was deduced from the results of $^1H$, and $^{13}C$ NMR and Mass Spectrometry. The product was separated using reverse phase HPLC, water/0.1% TFA and acetonitrile gradient using a Phenomenex C18 Gemini-NX 110 Å column (250 x 10.0 mm). BR-ACO-14 was synthesized following a published procedure.

### 1.1 UV/Vis spectroscopy: pH titration.

Twelve equimolar solution of **1** (20 μM) were prepared in phosphate buffer 0.1 M over a pH range of 4.2-10. Their absorption spectra were recorded on a Perkin Elmer Lambda 25 UV/Vis spectrophotometer in a 300-800 nm range at 20°C in a 1 cm path semi-micro plastic cuvette.

### 1.2 Fluorescence spectroscopy: pH titration.

Twelve equimolar solution of **1** (1 μM) were prepared in phosphate buffer 0.1 M over a pH range of 4.2-10. Their fluorescence emission spectra were recorded on a Horiba Jobin Ivon Ltd Fluorolog

Fluorometer. Upon excitation at 400 nm (first absorption band maximum) the emission spectra were recorded in a 415-700 nm range at 20°C in a 1 cm path quartz cuvette. The excitation and emission bandwidths were both fixed to 3 nm.

**1.3 Fluorescence spectroscopy: DNA titration.**

The following G-quadruplexes forming sequences were used: c-myc d(TGA GGG TGG GTA GGG TGG GTA A), c-kit1 d(AGG GAG GGC GCT GGG AGG AGG G), c-kit2 d(CCC GGG CGG GCG CGA GGG AGG GGA GG), k-ras d(AGG GCG GGT GTG GGA AAG AGG GAA AGA GGG GGA GG) and a duplex DNA: ss1 d(GGG CAT AGT GCG TGG CGT TTA GC) hybridized with its complementary sequence ss2 d(GCT AAA CGC CAC GCA CTA TGC CC). The DNA oligonucleotides were annealed in a buffer containing TRIS·HCl 50 mM pH 7.4 and KCl 100 mM, heating at 95°C for 5 min and then cooling to room temperature overnight.

Titration experiments with c-myc and duplex DNA: Aliquots of the concentrated preannealed solution of c-myc quadruplex (100 μM) and duplex DNA (50 μM) were added to the ligand solution (0.5 μM) ranging from 0 to 2.5 μM (0-5 equivalents). Fluorescence emission spectra were recorded on a Horiba Jobin Ivon Ltd Fluorolog Fluorometer immediately after the addition of the oligonucleotide to the ligand solution. Upon excitation at 400 nm (first absorption band maximum) the emission spectra were recorded in a 415-700 nm range at 20°C in a 1 cm path quartz cuvette. The excitation and emission bandwidths were both fixed to 5 nm.

**1.4 Surface Plasmon Resonance (SPR) experiments.**

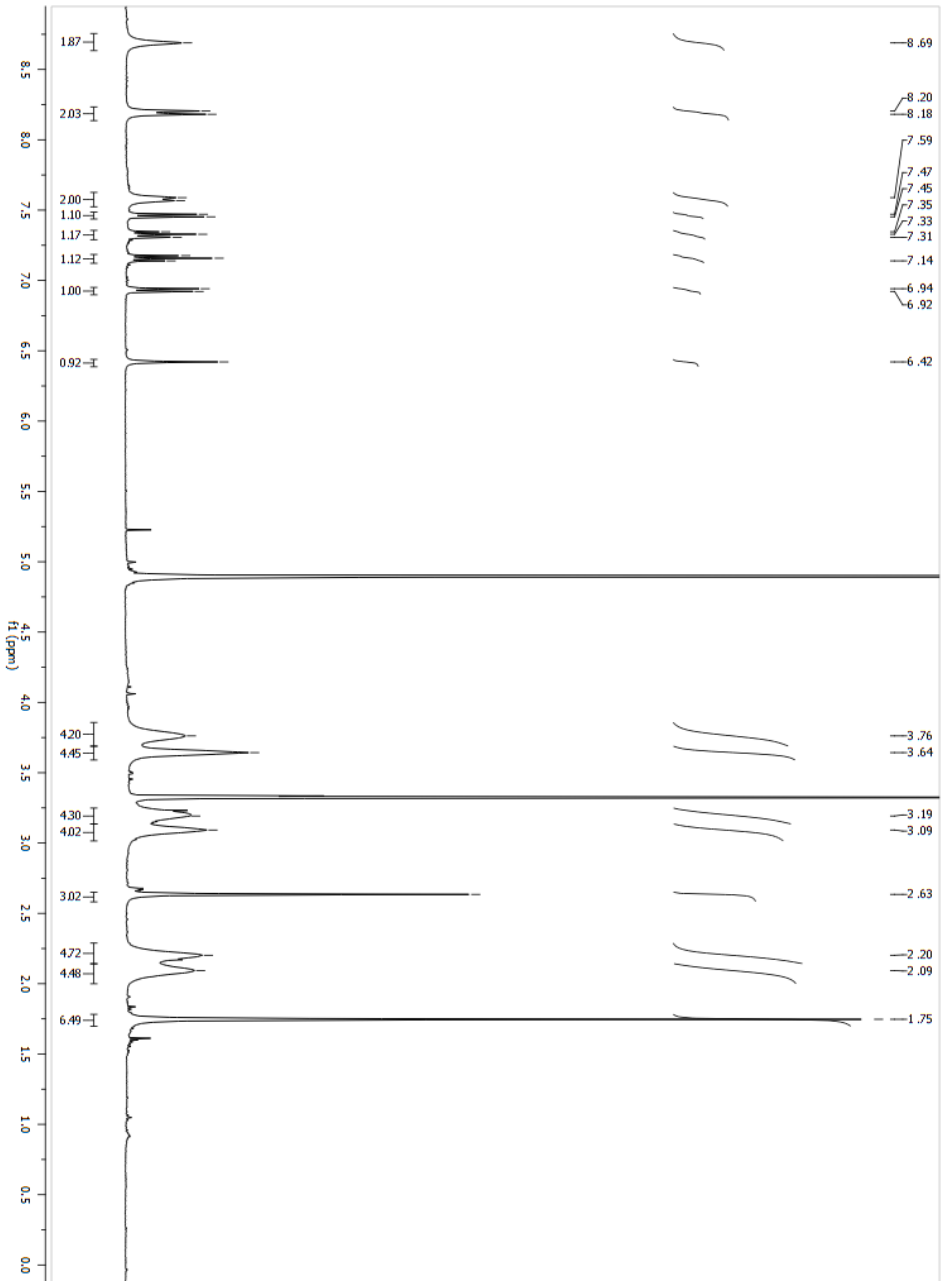
Surface plasmon resonance measurements were performed on a four-channel BIAcore 3000 optical biosensor system (Biacore Inc.) by using a streptavidin-coated sensor chip (Biacore SA-chip). The following biotinylated oligonucleotides were immobilized on individual flow cells: c-myc d(biotin-[TGA GGG TGG GTA GGG TGG GTA A]), c-kit2 d(biotin-[CCC GGG CGG GCG CGA GGG AGG GGA GG]), and a duplex DNA d(biotin-[GGG CAT AGT GCG TGG CGT TTA GC]) hybridised with its complementary sequence. The DNA oligonucleotides were folded in running buffer (Tris·HCl 50 mM pH 7.4, 100 mM KCl) by heating to 95 °C for 5 min and slow cooling to room temperature overnight. They were then immobilised (≈500 RU) except k-ras which was 2.5 times higher) in flow cells 1, 2 and 3, leaving the last flow cell empty as a blank. DNA binding experiments were carried out with running buffer at a flow rate of 20 $\mu L\,min^{-1}$. Solutions of ligands were freshly prepared in running buffer by serial dilutions from stock solutions. These solutions were injected by using the KINJECT command (Biacore 3000 Control Software version 3.0.1) for 2

min followed by a 30 s 1 M KCl injection and a 30 s running buffer injection for chip regeneration. Each sample injection was done is duplicate. The sensorgrams for the quadruplex and duplex DNA were corrected by subtracting the sensorgram obtained with the blank flow cell. The response at equilibrium (*Req*) was plotted against the concentration of analyte **1**. Dissociation constants were determined by fitting the binding curve by using the steady state affinity algorithm (Biaevaluation 3.0.2).

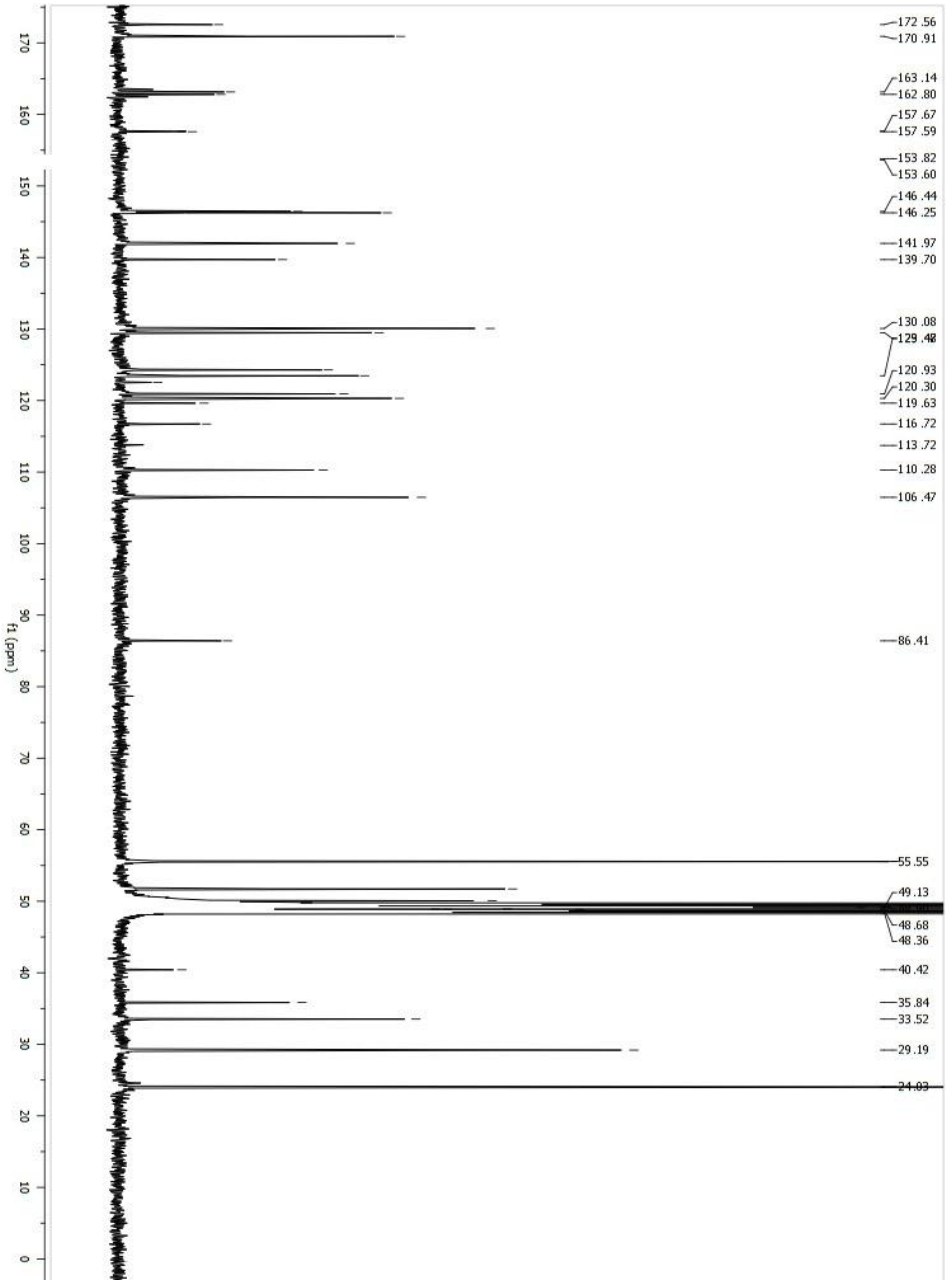
**1.5 Circular Dichroism experiments.**

CD spectra were recorded on a Jasco spectropolarimeter using a 1 mm path length quartz cuvette. c-myc oligonucleotide d(TGA GGG TGG GTA GGG TGG GTA A) (10 μM) was annealed in a TRIS·HCl buffer (50 mM pH 7.4) also containing 10 mM KCl by heating the solution at 95°C for 5 min and then slow cooling to room temperature overnight. The scans were performed at 10°C and 95°C respectively for two different solutions, in the presence and in the absence of 1 equivalent (10 μM) of ligand **1**. The scans were carried out over a wavelength range of 230-320 nm with a scanning speed of 100 nm/min. Two background spectra corresponding to either (i) the buffer alone or (ii) a solution of free ligand **1** (10 μM) in the same buffer were also recorded and subtracted from the collected data. The CD spectra represent an average of three scans.

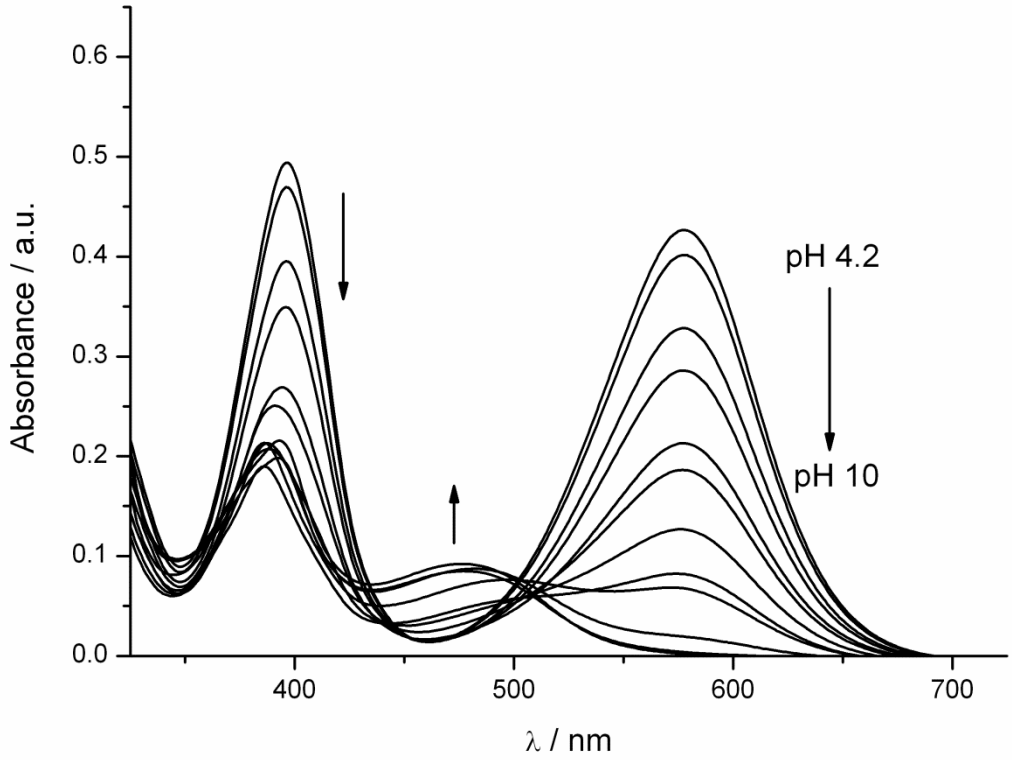
## $^{1}$H-NMR of (1):



## $^{13}$C-NMR of (1):

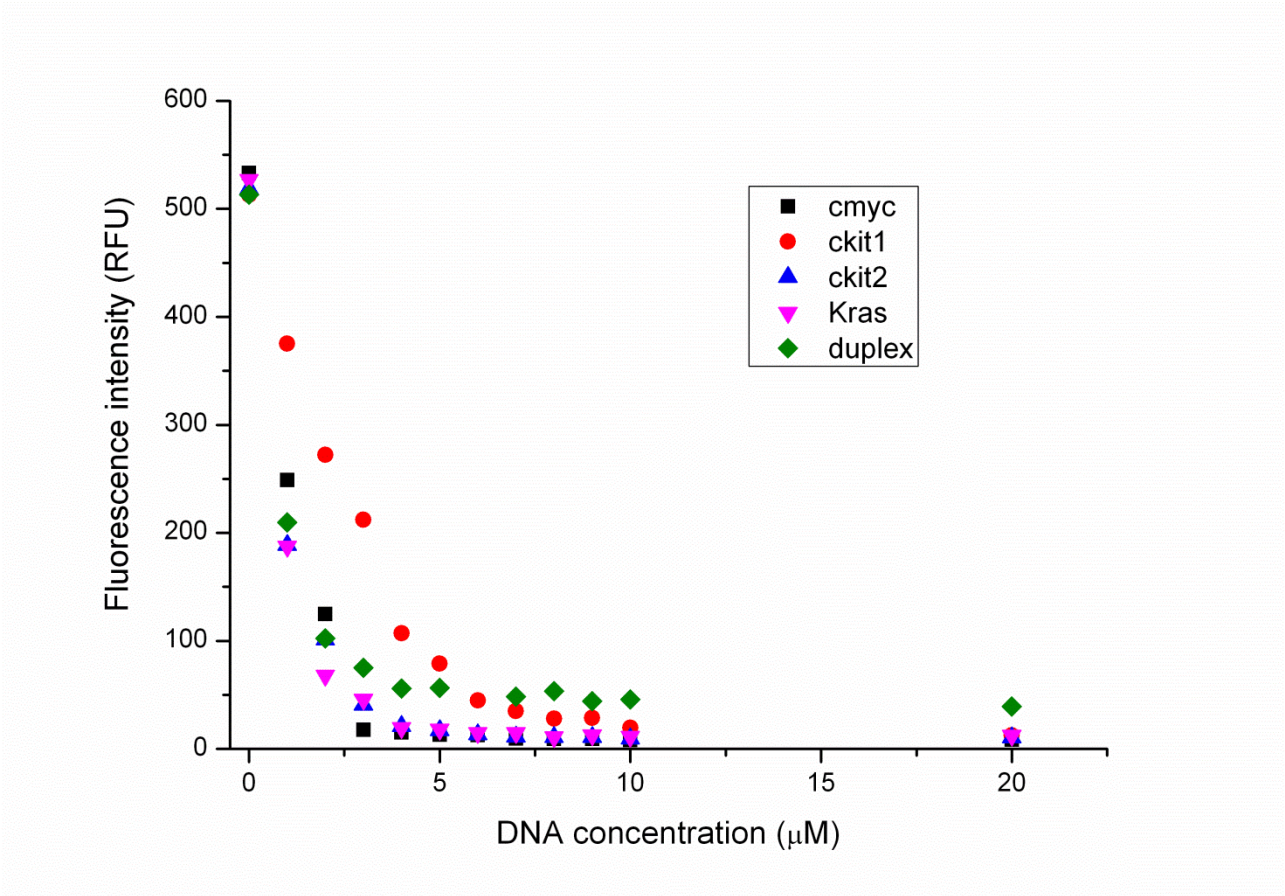


**3. UV-Vis pH titration experiment.**




Absorbance spectra of dye **1** in potassium phosphate buffers (100 mM) at various pH values ranging from 4.2 to 10. Dye concentration was fixed to 20 μM.

**3. DNA fluorescence titration experiments.**




A solution of dye **1** (10 μM) was titrated in with increasing concentrations of DNA (quadruplex and duplex, from 0 to 20 μM). Fluorescence was recorded using a fluorescence plate reader ($\lambda_{exc}$= 400 nm, $\lambda_{em}$= 475 nm).