Electronic Supplementary Material (ESI) for RSC Advances.

Selective Recognition of c-myc Promoter G-quadruplex and Down-Regulation of Oncogene c-myc Transcription in Human Cancer Cells by 3,8a-Disubstituted Indolizinone

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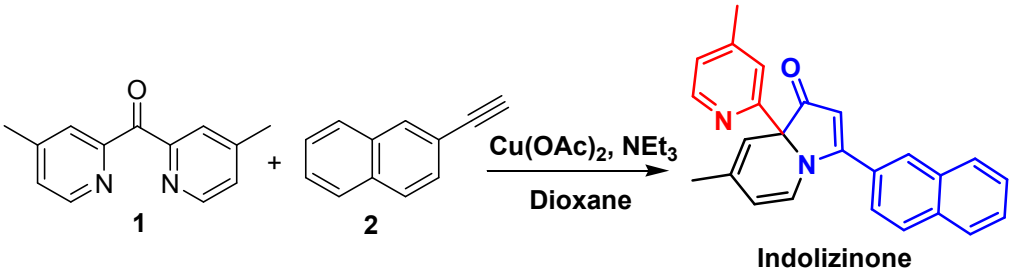
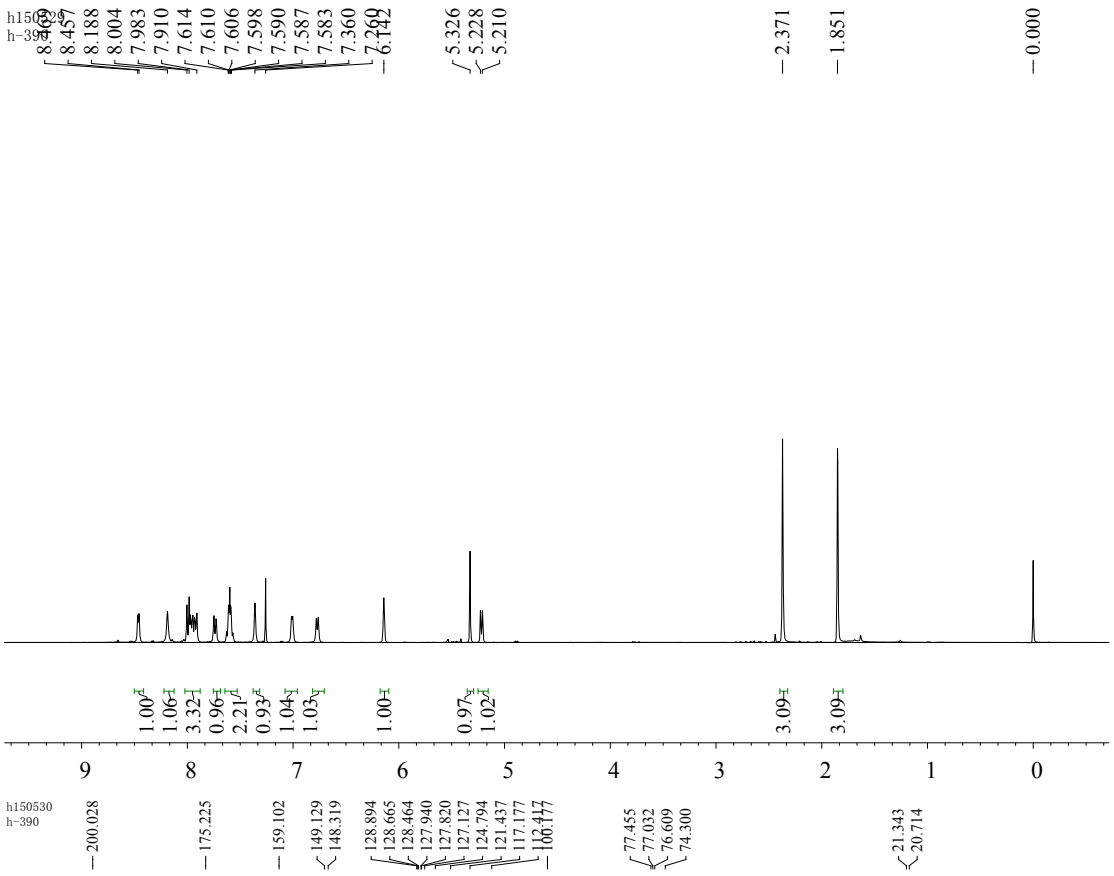
Preparation and characterization data of Indolizinone



To a screw-cap vial containing a stir bar, **1** (31.8 mg, 0.15 mmol), **2** (45.8 mg, 0.6 mmol), $Cu(OAc)_2$ (2.7 mg, 0.015 mmol) and NEt_3 (30.3 mg, 0.3 mmol) were added in 1,4-dioxane (1.5 mL). The reaction vial was fitted with a cap. The reaction vial was heated with stirring at 110 ^oC for 24 hours. After cooling down to room temperature and concentrated in vacuum, the residue was purified by flash chromatography on a short silica gel to provide the terminal product **Indolizinone** (45 mg, 82%, n-hexane/ethyl acetate = 3:1): yellow solid; ^1H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 4.8 Hz, 1H), 8.19 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 7.98 – 7.89 (m, 2H), 7.61 (d, *J* = 1.6Hz, 1H), 7.60 – 7.57 (m, 2H), 7.36 (s, 1H), 7.01 (d, *J* = 4.0 Hz, 1H), 6.77 (d, *J* = 7.2 Hz, 1H), 6.14 (s, 1H), 5.33 (s, 1H), 5.22 (d, *J* = 7.2 Hz, 1H), 2.37 (s, 3H), 1.85 (s, 3H).; ^{13}C NMR (100 MHz, CDCl3) δ 200.0, 175.2, 159.1, 149.1, 148.3, 134.3, 132.9, 132.1, 128.9, 128.7, 128.5, 127.9, 127.8, 127.1, 127.0, 124.8, 124.2, 123.7, 121.4, 117.2, 112.4, 100.2, 74.3, 21.3, 20.7.; HRMS (ESI): Exact mass calcd for $C_{25}H_{21}N_2O^+$ $[M+H]^+$ 365.1645, found 365.1648.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6.142
5.326
5.228
5.210
2.371
1.851
0.000
1.00
1.06
3.32
0.96
2.21
0.93
1.04
1.03
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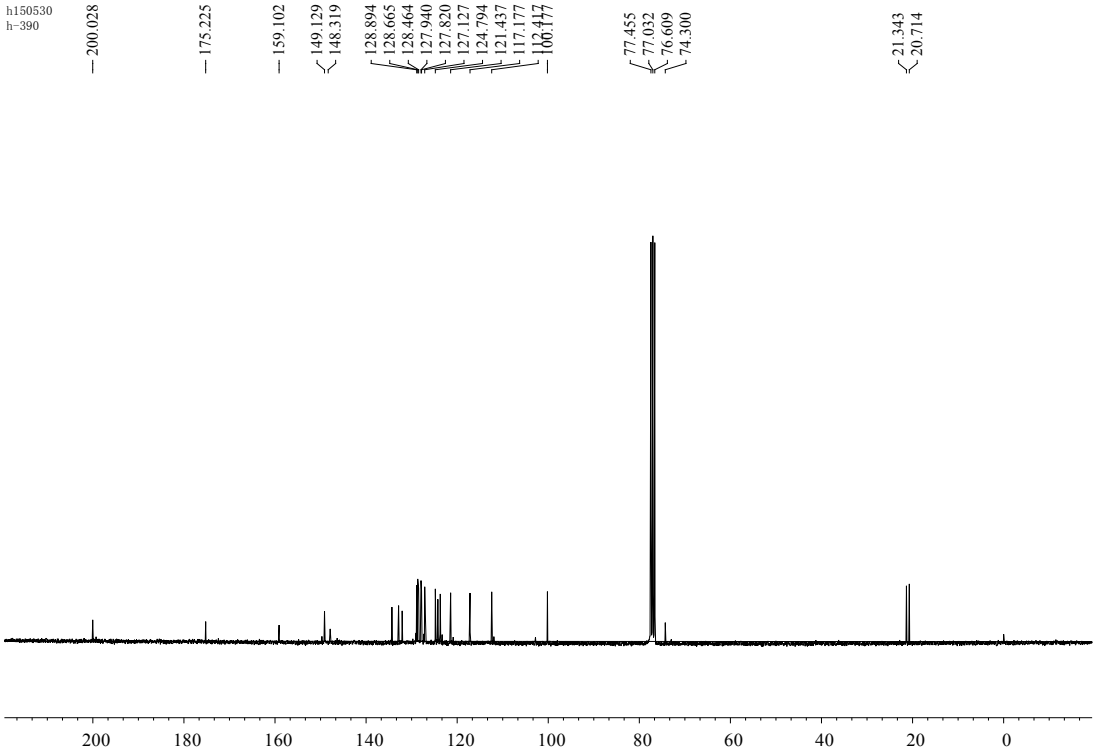
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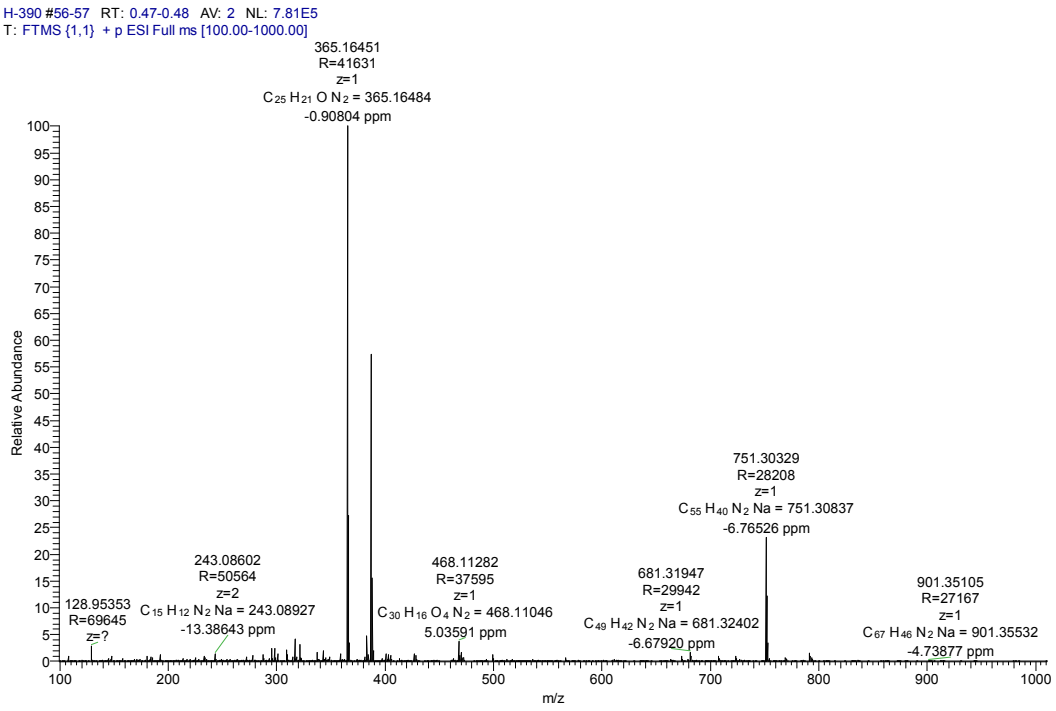

Sample preparation for CD spectra and Fluorescence spectra

All the DNA oligonucleotides were purchased from ZixiBio Co. Ltd., purified by PAGE. All the samples were measured in Tris-HCl (40 mM KCl, pH 7.0) solution.

Spectroscopy measurement

CD spectra were collected from 200 to 350 nm on a Jasco-815 automatic recording spectro polarimeter with a 1-cm pathlength quartz cell at 25 ^{o}C. Spectra were collected with scan speed of 500 nm min^{-1}. Each spectrum was the average of three scans.

The addition of Indolizinone did not cause a transition of the CD spectra of all the G-quadruplexes investigated, indicating that the conformations of all the G-quadruplexes were not altered, which facilitates the study on the interactions between Indolizinone and G-quadruplexes.

Fluorescence spectra were recorded on a Hitachi F4500 spectrofluorometer (Japan) in a 1 cm pathlength quartz cell at room temperature. Xenon arc lamp was used as the excitation light source. The excitation and emission slits were both 10 nm. Excitation was set at 423 nm, and emission was collected from 440 to 650 nm.

Table S1. Oligonucleotides used in Table 1 in the manuscript.

Name	Type / origin	Sequence(from 5'to 3')
Myc	G4-Promoter	TGAG$_3$T G$_3$GA G$_3$TG$_3$GAA
Kit	G4-Promoter	G$_3$AG$_3$CGCTG$_3$AGGAG$_3$
A24	G4-HumanDNA telomere	TTA G$_3$TTA G$_3$TTA G$_3$TTA G$_3$

M24	G4-HumanDNA telomere	TTA G_3TTA G_3TTA G_3TTA G_3A
H7	G4-HumanDNA telomere	TTA G_3T

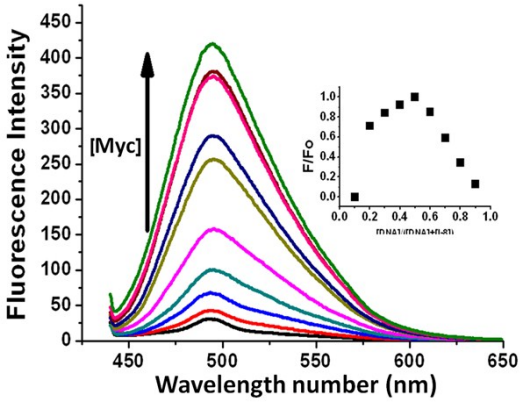

Figure S1. Fluorescence titration of 4 μM Indolizinone with increasing amounts of DNA MycG4 in Tris-HCl (40 mM KCl, pH 7.0) solution. Upper insertion is the Job's plot.

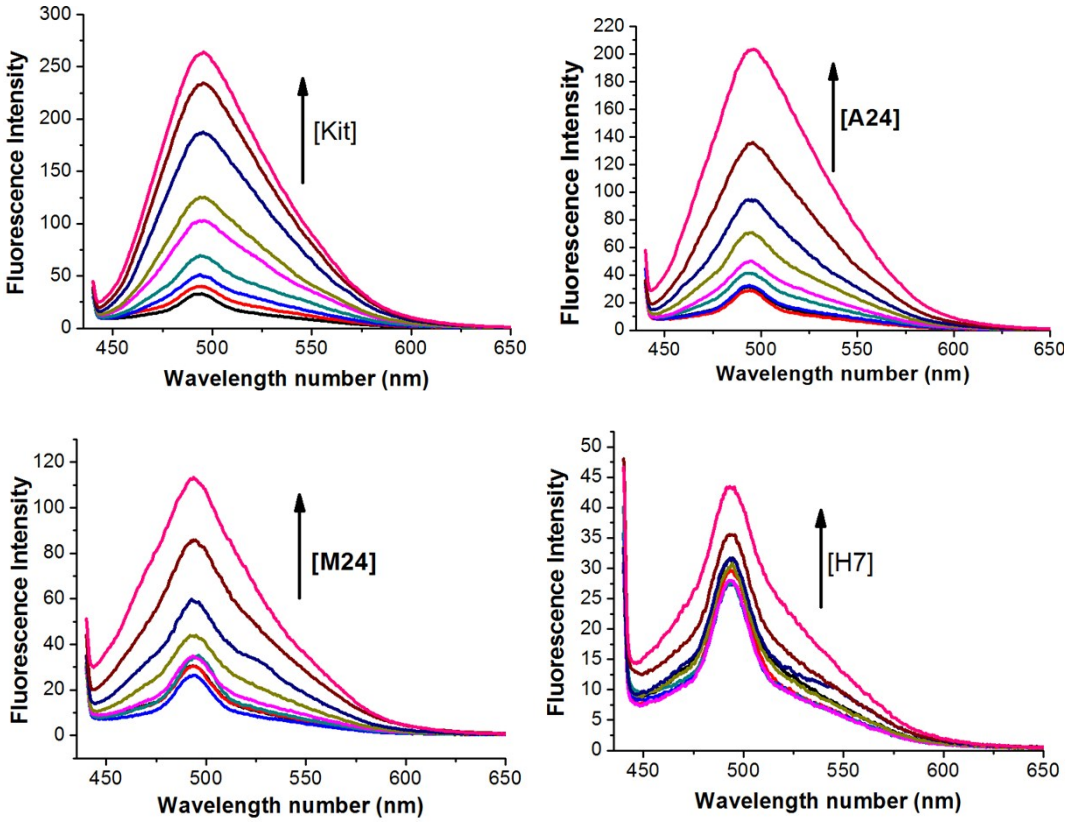

Figure S2. Fluorescence titration of 4 μM Indolizinone with increasing amounts of DNA G-quadruplexes in Tris-HCl (40 mM KCl, pH 7.0) solution.

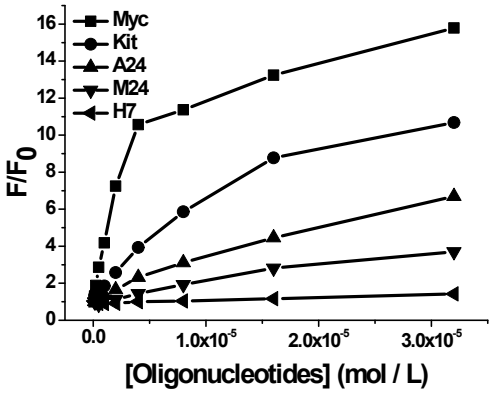

Figure.S3. Variations of fluorescence intensity F/F_0 of Indolizinone (4 μM) with increasing varied five DNA G-quadruplexes in Tris-HCl (40 mM KCl, pH 7.0) solution.

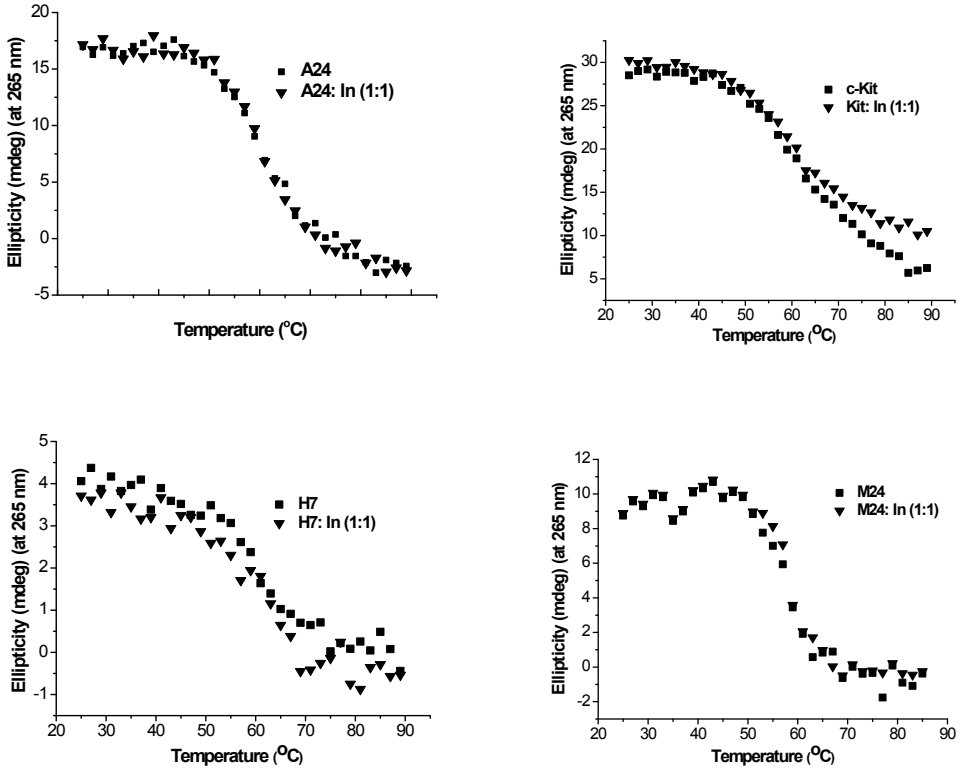

Figure S4. Varied-temperature CD melting profiles of different DNA G-quadruplexes in the absence or presence of Indolizinone (1 eq.)

RT-PCR Experiment

The total RNA samples were isolated using TRIzol (Life Technologies, China). cDNA samples were synthesized using the Prime Script RT Master Mix (B-Belife, China). The real-time PCR analyses were performed using SYBR Premix Ex Taq Ⅱ (B-Belife, China). The sequences of c-myc primers were as follows: 5'- GGACTTGTTGCGGAAACGAC -3' and 5'- CTCAGCCAAGGTTGTGAGGT -3'. The sequences of Beta actin were as follows: forward, 5'- TGACGTGGACATCCGCAAAG -3'; reverse, 5'- CTGGAAGGTGGACAGCGAGG -3'. Beta actin was used as an internal control for the relative

quantitation of mRNAs. All experiments were performed in triplicate. Relative quantification of gene expression was performed by the $2^{-\Delta\Delta Ct}$ method.