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PAPER

Synthesis of phenanthiridine spiropyrans and studies of their effects on G-quadruplex DNA

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G-quadruplex (G4) DNA structures are involved in many important biological processses and can be linked to several human diseases. Drug-like low molecular weight compounds that target G4 structures are therefore interesting for their potential therapeutic properties but also for their potential use as chemical research tools. We report here on the development of methods to synthesize spiropyrans using a condensation-cyclisation reaction of quaternary salts of α-methyl quinoline or phenanthridine with salicylaldehydes. Evaluation of the synthesized phenanthridine spiropyrans interactions with G4 DNA was performed with a ThT displacement assay, circular dichroism (CD), Taq DNA polymerase stop assay, and NMR. This revealed that the substitution pattern on the phenanthridine spiropyrans was very important for their ability to bind and stabilize G4 structures. Some of the synthesized low molecular weight spirocyclic compounds efficiently stabilized G4 structures without inducing structural changes by binding the first G-tetrad in the G4 structure.

Introduction

Guanine-rich sequences of single stranded DNA or RNA can fold into secondary DNA structures called G-quadruplex (G4) structures. The G4 structures are composed of two or more stacks of guanines (G-tetrads) that align on top of each other. Each G-tetrad is composed of four guanines that interact through Hoogsteen hydrogen bonds,[1] and are further stabilized by a central monovalent cation, such as potassium or sodium.[2] Although this central arrangement is similar for all G4 structures, their overall structure can differ significantly, as the structures can form inter- or intra-molecularly, and comprise of different number of guanine stacks. Also the nucleotides bridging the central guanines (known as loops) can differ, and the sequences that build up the G4 structure can be in parallel, antiparallel, or hybrid orientations.[3]

The evolutionary conserved genomic locations of many G4 sequences suggest that they are involved in biological functions, such as gene regulation, DNA replication, and telomere maintenance.[4] G4 sequences are also associated to cancer and a variety of other human diseases.[5] The number of sequences with potential to form a G4 structure in the human genome is immense, but which sequences that form a G4 structure *in vivo* and their biological functions are still not fully elucidated.

Low molecular weight compounds that target G4 structures have great potential as drug candidates and as chemical research tools.[4c, 6] We recently performed a high-throughput screen (HTS) with over 28,000 compounds, which identified new G4-binding small molecules.[7] Among these structures was a phenanthridine based spiropyran compound (**7**, Table 1). Spirocycles are a common moiety in many natural products and frequently appear in drug development.[8] Spirocyclic compounds are structurally interesting as they have an intrinsic three-dimensional feature, which is beneficial compared to the many flat aromatic compounds used as lead compounds in medicinal chemistry in general,[9] and as G4 stabilizing compounds in particular.[4c, 10] Here, we report the development of synthetic methods to phenanthridine spiropyrans that were used to synthesize over 20 analogues. The abilities of these low molecular weight spirocyclic compounds to interact with G4 structures were evaluated in orthogonal assays. This not only revealed compounds with high binding and stabilization efficiencies, but also information about how the compounds bind to the G4 structure.

Results and discussion

Synthetic procedures

A previous report on the synthesis of these types of central fragments inspired us to begin our investigation by developing a general reaction between quaternary salts of α-methyl quinoline and phenanthridine in a condensation, cyclisation reaction with different salicylaldehydes.[11]

To probe the viability of the synthetic steps we initially made the triflate salt of 1,2-dimethylisoquinolin-2-ium (**1**) by methylation of commercially available 1-methylisoquinoline

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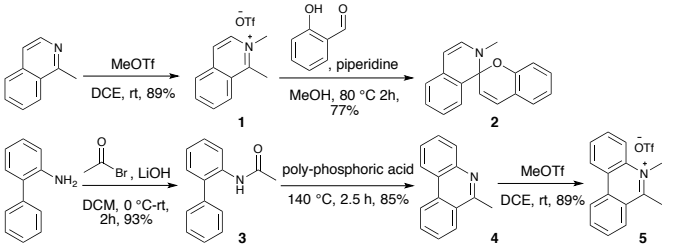


Scheme 1. Synthesis of spiropyran **2** and phenanthridinium salt **5**

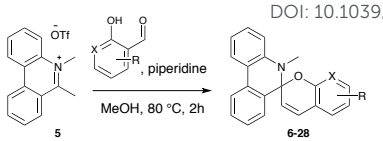
and reacted it with salicylaldehyde. This led to the isolation of spiropyran **2** in 77% yield (Scheme 1). Inspired by this, we started to develop the synthesis of the triflate salt of 5,6-dimethylphenanthridin-5-ium (**5**), as this was structurally more closely related to our hit compound. The synthesis was accomplished in three steps starting with acetylation of commercially available 2-aminobiphenyl to give **3** followed by a Bischler-Napieralski reaction generating the phenanthridine core in **4**. The nitrogen was then easily methylated using methyl triflate at room temperature resulting in the desired quarternary salt **5** (Scheme 1).

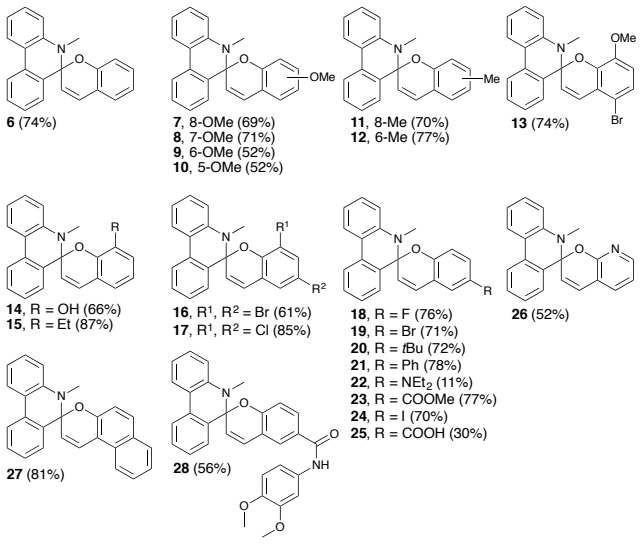
The final spiropyran products were obtained through a one-pot condensation, cyclisation reaction between triflate salt **5** and different salicylaldehydes (Table 1, top). Key to the success of the reaction proved to be the portion-wise addition of the triflate salt to drive the complete consumption of the salicylaldehyde. In almost all cases the product precipitated out of the reaction, driving the equilibrium of the reaction towards product. The products were isolated in high yields from the reaction mixtures through filtration. By using this method, over 20 phenanthiridine spiropyrans were synthesized with both mono- and di-substitutions in different positions on the benzopyran ring system. A wide range of substituents proved to be tolerated in the reaction such as halides, *tert*-butyl, phenyl, methoxy, amide, methyl ester, and even a carboxylic acid substituent, albeit the latter resulted in a lower yield (Table 1).

In the cases where no precipitation of the product occurred, for example in the synthesis of phenanthridine spiropyrans **14**, **22**, and **26**, the isolation was more complicated because of the formation of ring-opened spiropyran merocyanine forms, which also resulted in lower yields. The ring-opening to the merocyanine form was not induced by near UV light but seem to be substrate dependent.

These synthetic methods, both for the triflate salt **5** as well as the following spiropyran formation, present efficient routes towards these types of scaffolds. The routes are amenable to a high degree of variation both in the salicylaldehyde part as well as in the quaternary salts.

Table 1. Synthesis of spiropyrans[a]





[a] Reaction conditions: Phenanthridinium salt **5** (1 equiv.) was added portion-wise to salicylaldehydes (1 equiv.) and piperidine (1 equiv.) in refluxing methanol (0.1 M) over 2h. Trace amounts of ring-opened merocyanine forms are present in **14** and **22**.

Compound evaluation

To investigate the compounds abilities to bind G4 structures, we used a Thioflavin T (ThT) displacement assay (see supplemental information).[7, 12] Using this assay we tested binding of the different analogues to a known G4 structure forming oligonucleotide, i.e., the ribosomal DNA (rDNA) sequence from *Schizosaccharomyces pombe* (Figure 1A), which forms a parallel-stranded G4 structure.[13] To calculate the half maximum displacement concentration (DC_{50}), the compounds were tested at 16 different concentrations ranging from 0.19 nM to 50 µM (Figure 1B, top).

The structural variations in the synthesized analogues resulted in large variations in their activity in this assay and several compounds demonstrated similar or even improved activities compared to the original hit structure (**7**). The most active compound in the series was the 7-methoxy substituted phenanthiridine spiropyran **8** with a DC_{50} value of 0.2 µM. The following regioisomer, the 6-methoxy analogue **9**, had a slightly higher DC_{50} value, about two-fold, but was still submicromolar. Interestingly the following regioisomer, the 5-methoxy analogue **10**, showed almost 11-fold reduced binding compared to the hit structure with a DC_{50} value of 3.3 µM. The

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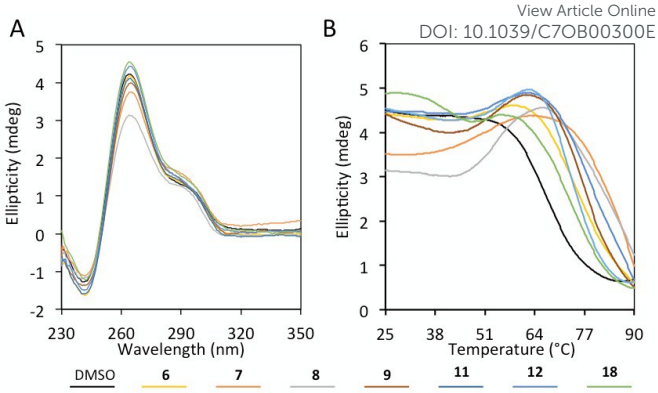
A *S. pombe* rDNA (25 bp): GGGGAAGGGTGGGGCATGTTATGGG
Human Pu24T-c-*MYC* (24 bp): TGAGGGTGGTGAGGGTGGGGAAGG

B

compound	6	7	8	9	10	11	12	13	14	15	16	17
rDNA	0.5	0.3	0.2	0.4	3.3	0.6	0.8	1.1	1	1.5	3.1	1.2
compound	18	19	20	21	22	23	24	25	26	27	28	-
rDNA	0.9	4.4	>10*	>10	1	>10*	5.4	7	6.9	1.1	5	-
compound	6	7	8	9	11	12	18	-	-	-	-	-
c-*MYC*	>10*	0.5	0.7	1	1.5	>10*	>10*	-	-	-	-	-

Figure 1. ThT displacement assay. (A) Oligonucleotide templates used in the ThT displacement assay. (B) DC_{50} values of ThT displacement (μM) with a DNA concentration of 1 μM. *Estimated DC_{50} values as the ThT signal was not completely reduced at the highest compound concentrations.



C

Compound	6	7	8	9	11	12	18
Estimated ΔT_m	+9	+17	+17	+11	+8	+13	+7

Figure 2. The melting temperature is increased in the presence of phenathridine spiropyran compounds. (A) CD spectra of 5 μM rDNA supplemented with 1.25% (v/v) DMSO and 80 μM compounds at 25 °C. (B and C) Melting curves of the same samples were recorded at 264 nm at temperature range 25-90 °C. T_m corresponds to the estimated value when 50% of the DNA is melted. T_m of the rDNA treated with 1.25% (v/v) DMSO is 68 °C. ΔTm describes T_m difference between T_m of the DNA treated with compounds compared to DMSO. All the data are shown in °C. The reason to the initial increase in ellipticity is examined in supporting figure S2.

positioning of the substituents on the benzopyran ring system is thus of key importance for the activity. Furthermore, the methoxy substituent is preferred over the structurally related 8-methyl substituted analogue **11**, 8-hydroxy analogue **14**, and 8-ethyl substituted analogue **15**, which have about 2-, 4-, and 5-fold less efficient binding than **7**, respectively.

Generally, the most efficient binders in this assay have small substituents, such as methyl, methoxy or fluorine in position 6, 7, or 8 on the benzopyran ring system. Larger substituents such as the 6-*tert*-butyl in analogue **20**, or the 6-phenyl in analogue **21** showed significantly reduced activity, with DC_{50} values over 10 μM. Methyl ester **23**, benzoic acid **25** and amide **28** all had fairly high DC_{50} values ranging from around 5 to over 10 μM whereas the 6-diethylamino analogue **22** had a DC_{50} value of around 1 μM. The pyridine analogue **26** was not very active in this assay, with a DC_{50} value of around 7 μM, potentially because of the difference in electron density and basicity induced by the heteroaromatic ring. The addition of a second 5-bromo-substituent to the benzopyran ring system of phenathridine spiropyran **7**, as in the 8-methoxy-5-bromo substituted analogue **13**, reduced the activity and resulted in a DC_{50} value of 1.1 μM. The 8,6-dibromo and 8,6-dichloro substitutions in analogues **16** and **17** resulted in DC_{50} values of 3.1 μM and 1.2 μM, respectively.

Additionally, we also performed the ThT displacement assay with the Pu24T *c-MYC* promoter sequence (Figure 1A),[14] which also forms a parallel-stranded G4 structure, with our seven best binders **6**, **7**, **8**, **9**, **11**, **12** and **18.** Also with this G4 structure, the methoxy substituent (as in **7**, **8**, and **9**) proved to be highly important for a low DC_{50} value as well as the positioning of the substituents in position 7 or 8 on the benzopyran ring system (compare phenanthridine spiropyran **7** versus **9** and **11** versus **12** in Figure 1B, bottom). Overall, the compounds' binding efficiency for this G4 structure were slightly lower than with the rDNA G4 structure, but showed the same relative trend.

To investigate if the compounds induce a structural change of the G4 structure upon binding, circular dichroism (CD) spectroscopy was used. The most active compounds from the ThT displacement assay **6**, **7**, **8**, **9**, **11**, **12**, and **18** were evaluated with the rDNA G4 structure at 8:1 (compound:DNA) molar ratios (Figure 2A). None of the tested compounds induced any significant structural change in the G4 structure, which is beneficial from a research tool perspective and is also in contrast to the large structural changes induced by many of the most frequently used G-quadruplex stabilizing compounds today.[15] The absorbance spectra for compounds **6**, **7**, **8**, **9**, **11**, **12**, and **18** are displayed in supporting information figure S1.

Further, the ability of these compounds to stabilize the rDNA G4 structure was also studied by using CD spectroscopy and stepwise increasing the temperature (Figure 2B and C). All of the tested phenanthridine spiropyrans stabilized the rDNA G4 structure. Analogues **7** and **8** resulted in the strongest stabilization with an increased estimated G4 structure melting temperature (T_m) of +17 °C. In accordance with the observations in the ThT displacement assay, moving or replacing the methoxy substituent in **7** and **8** substantially reduced the G4 stabilization capacity of the compounds. The 6-methoxy analogue **9** increased the T_m with +11 °C compared to +17 °C for both the 7- and 8-methoxy analogues **7** and **8**. Replacing the methoxy substituent with a methyl group as in **11** and **12** or with a fluorine as in **18** increased the G4 stabilization with +8 °C, +13 °C, and +7 °C, respectively. Removing the methoxy substituent as in analogue **6** reduced the G4 stabilization from +17 °C to +9 °C. Some of the compounds resulted in an initial increase in ellipticity. This maybe due to the existence of both a parallel and antiparallel structural motifs in the rDNA G4 structure, which unfolds and folds differently in the presence of the compounds (supporting information figure S2).

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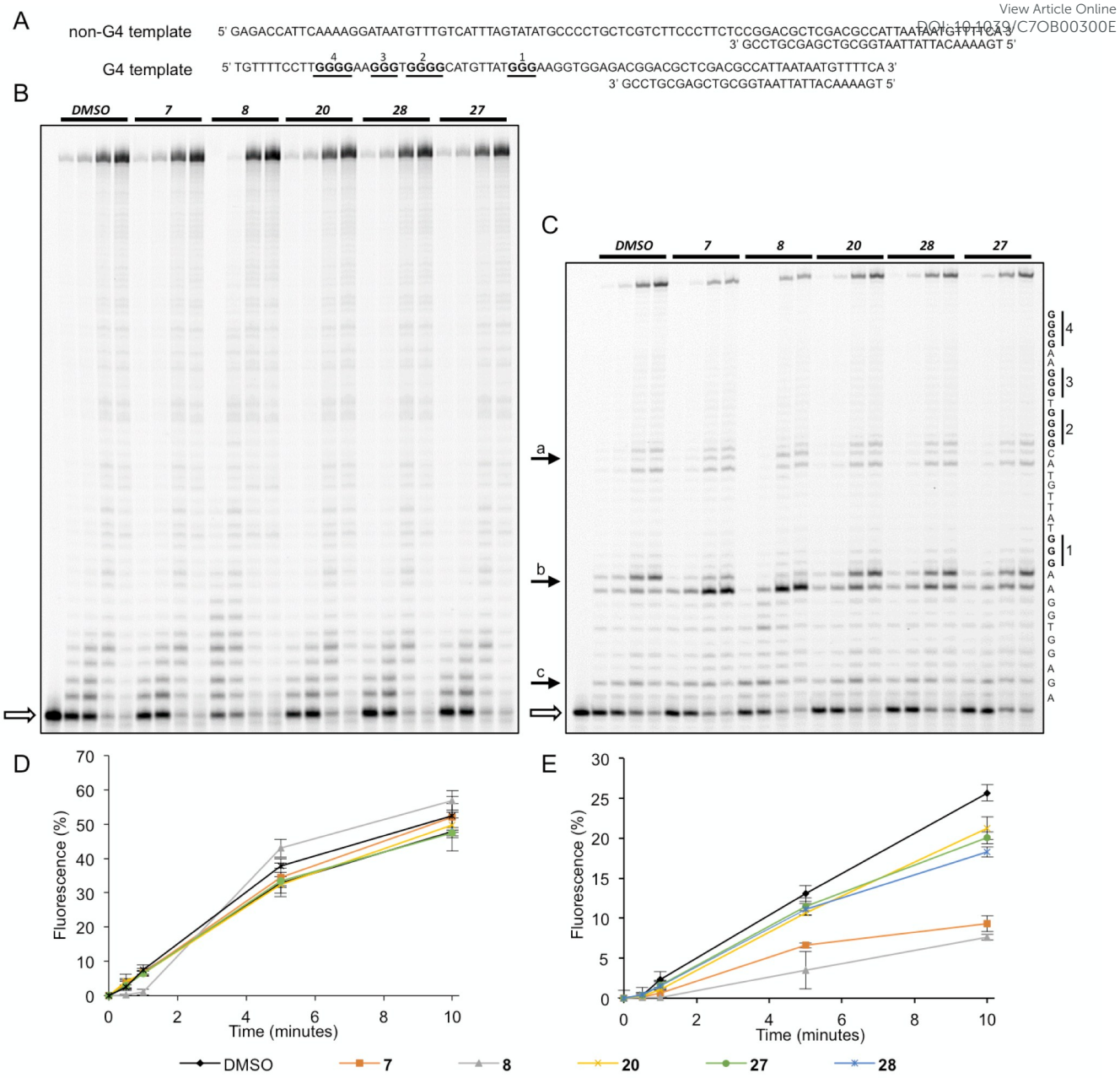

Figure 3. Compounds enhance pausing of Taq polymerase at rDNA G4 structure. (A) Oligonucleotide sequences of the primer-templates used in the primer extension assay. The sequence of the non-G4 control template is from a region from the *S. pombe ade6*$^{+}$ gene and the sequence of the G4 template is from the S. pombe rDNA region. The G-tracts are numbered, underlined, and shown in bold. (B) Primer extension assay using the non-G4 template in the presence of DMSO, 25 μM of compound 7, compound 8, compound 20, compound 27, and compound 28. Each reaction was initiated by addition of the Taq polymerase and incubated at 50 °C for either 0.5, 1, 5 or 10 minutes. Open arrow indicates the position of the primer. (C) Primer extension assay was carried out with the G4 template and run as described in B. The first nucleotide of the single-stranded region of the primer-template until the last G-tract region is shown on the right side of the gel. The G-tracts are in bold and numbered as in A. Black arrows indicate the pausing sites labeled as a, b and c upstream GGG-tract 2, GGG-tract 1, and the GG-tract. (D) and (E) Quantification of Taq DNA polymerase stop assay. Graphs are showing the average of the percentage of full-length products of the ade6+ synthesis in A and rDNA synthesis in B, quantified from two independent experiments. Error bars represent absolute error.

The effect of the changes in the benzopyran ring system of the compounds was also examined by the Taq DNA polymerase stop assay (Figure 3). In this assay, stabilization of the DNA results in an enhanced pausing of the DNA polymerase, which result in a reduced amount of full-length product (measured after 0.5, 1, 5, and 10 minutes reactions). We used a 5' fluorescently labeled primer annealed to the rDNA G4 template or a non-G4 template from *S. pombe ade6*$^{+}$ gene as a control (Figure 3A).

First, we examined the effect of the compounds on the primer extension of non-G4 DNA. We observed similar increase of the full product in all the samples, treated either with compounds **7, 8, 20**, **27,** and **28** or DMSO (Figure 3B and D). An increased termination probability in the early time

points (0.5 and 1 min) was observed with compound **8** but these changes were not present in the later time points (5 and 10 min).

Next, we examined if the compounds stabilized the rDNA G4 structure and thus blocked DNA synthesis. If so, the amount of full-length product should be significantly decreased and most of the signal should be detected at the pausing sites before the G-tracts. Phenanthridine spiropyrans **7** and **8** significantly stabilized the rDNA G4 structure and reduced the amount of full-length product almost three-fold after 10 min incubation (Figure 3C and E). Pausing by the DNA polymerase caused by **7** and **8** was observed two nucleotides upstream of the first and second G-tract (Figure 2, arrow a and b). In addition, the small structural difference between **7** and **8** resulted in the enrichment of another pausing site for **8**, two nucleotides upstream of another G-tract, containing only two guanines, instead of three guanines (Figure 3C, arrow c). Pausing at this site was also detected in an earlier study and may suggest that this region also is part of the G4 structure.[7] As expected, the phenanthridine spiropyrans **20**, **27,** and **28** did not display any large stabilization of the G4 structure, which is in alignment with the DC_{50} values (Figure 1B).

NMR analysis

To understand how the compunds interact with G4 DNA we evaluated compounds **7** and **8** by using ^{1}H-NMR. Spectra were recorded on 100 μM folded Pu24T *c-MYC* G4 structure (that also was used in the ThT assay, Figure 1B, bottom) in absence and presence of equimolar amounts of either **7** or **8**. Analysis of the imino-region of the spectra showed line-broadening of most guanine imino peaks in the presence of both **7** and **8** (Figure 4). In particular, the signals from the guanine bases in positions 4, 8, 13, and 17 were broadened to such a degree that they disappeared in the baseline. These guanine bases form the first G-tetrad in this particular G4 structure, which indicate that the top tetrad is involved directly in the binding of **7** and **8**. Similar effects have been reported in previous studies of small molecules binding to Pu24T *c-MYC* G4 DNA.[14, 16]

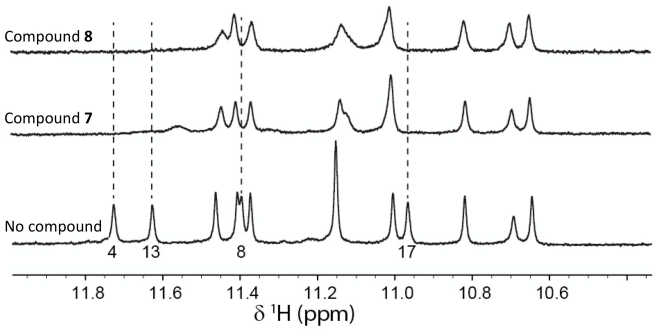

Figure 4. The imino-region of ^{1}H spectra recorded for Pu24T *c-MYC* G4 DNA in absence (bottom) and presence of compound **7** (middle) and **8** (top). Imino protons from guanine bases in the top tetrad are marked with numbers and dashed lines.

Conclusions

We have developed synthetic methods to phenanthridine spiropyrans that are applicable to a wide range of substituents. The compounds ability to bind and stabilize G4 structures proved to be highly dependent on the substitution pattern on the spiropyrans. Methoxy substituents in position 7 or 8 in the benzopyran ring system resulted in efficient G4 structure stabilization without induction of structural changes, by binding to the first G-tetrad in the G4 structure. The acquired binding information and structure activity relationships is of importance for future optimizations of this low molecular weight spirocyclic class of compounds and also for their use and further development as chemical research tools.

Experimental

Synthesis

General Synthesis. Unless stated otherwise, all reagents and solvents were used as received from commercial suppliers. 3-Ethyl-2-hydroxybenzaldehyde was prepared according to a previously published procedure.[17] TLC was performed on aluminum backed silica gel plates (median pore size 60 Å, fluorescent indicator 254 nm) and detected with UV light. Flash column chromatography was performed using silica gel with an average particle diameter 50 μm (range 40–65 μm, pore diameter 53 Å), and eluents are given in brackets. DMF, THF and DCM were dried in a solvent drying system (THF and DCM drying agent: neutral alumina ; DMF drying agent: activated molecular sieves, also equipped with an isocyanate scrubber) and were collected fresh prior to every reaction. 1H and 13C NMR spectra were recorded on a Bruker 400 MHz spectrometer at 298 K or on a Bruker 600 MHz spectrometer at 298 K, and calibrated by using the residual peak of the solvents as the internal standard (CDCl3: δ H= 7.26 ppm; δ C = 77.16 ppm. DMSO-d6: δ H = 2.50 ppm; δ C = 39.50 ppm). LC-MS was conducted on an Agilent 6100 Series Quadropole LC/MSD system . HRMS was performed by using a Agilent 1290 binary LC System connected to a Agilent 6230 Accurate-Mass TOF LC/MS (ESI+); calibrated with Agilent G1969-85001 ES-TOF Reference Mix containing ammonium trifluoroacetate, purine and hexakis(1H, 1H, 3H-tetrafluoropropoxy)phosphazine in 90:10 acetonitrile:water. Preparatory HPLC was performed on a C18 reversed-phase column (25 cm x 21.2 mm, 5 mm) with H2O/MeCN mixtures as the eluent.

Abbreviations. DMF – dimethylformamide, MeOH – methanol, THF - tetrahydrofuran, EtOAc – ethyl acetate, TFA – trifluoro acetic acid, DCE – dichloroethane, DCM – dichloromethane, MeCN – acetonitrile, DMAP – dimethylaminopyridine, TLC – thin layer chromatography, HPLC – high pressure liquid chromatography, LCMS – liquid chromatography mass spectrometry, rt – room temperature

1,2-dimethylisoquinolin-2-ium trifluoromethanesulfonate (**1**): Methyltrifluoromethansulfonate (307 μl, 2.71 mmol) was added to a solution of 1-methylisoquinoline (360 μl, 2.71 mmol) in 1,2-dichloroethene (5 ml). The reaction mixture was stirred at rt for 5 h. A white solid precipitated from the

solution. After filtering off the solid, it was washed with cold diethyl ether (0 °C) and dried under reduced pressure. The product was isolated as a white solid in 87 % yield (722 mg, 2.35 mmol) ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.77 (d, J = 8.7 Hz, 1H), 8.68 (d, J = 6.9 Hz, 1H), 8.38 (d, J = 6.9 Hz, 1H), 8.29 (d, J = 8.1 Hz, 1H), 8.21 (t, J = 7.1 Hz, 1H), 8.04 (ddd, J = 8.4, 6.9, 1.3 Hz, 1H), 4.38 (s, 3H), 3.21 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 160.6, 136.6, 136.5, 135.8, 130.9, 128.4, 127.9, 127.1, 123.4, 121.1 ($J_{C\text{-}F}$ = 321 Hz), 46.4, 16.8. LCMS: Calculated M^+ (cationic part only) (158); Found (M^+) 158 m/z, Rt = 1.29 min.

2'-Methyl-3,8a-dihydro-2'H-spiro[chromene-2,1'-isoquinoline] (**2**) (ECH23): Made using the general procedure (*vide infra*) from 1 (100 mg, 0.33 mmol) and salicylaldehyde (34 µl, 0.33 mmol).The crude mixture was concentrated and purified by preparative HPLC using MeCN:water with 0.75% formic acid as eluent, giving the open zwitterionic intermediate after condensation with the aldehyde. The isolated intermediate was then dissolved in DMSO and sodium carbonate (35 mg, 0.33 mmol) was added to the solution. The suspension was stirred at rt for 12 h. The reaction mixture was diluted with dichloromethane and washed with water three times. The organic layer was dried with sodium sulfate and the solvent was removed under reduced pressure. The product was a red solid. Yield: 77 % (65 mg, 0.25 mmol).1H-NMR (400 MHz, DMSO-d6): δ = 7.27 (t, J = 7.2 Hz, 1H), 7.24 (d, J = 7.0 Hz, 1H), 7.23 (t, J = 7.3 Hz, 1H), 7.16 (d, J = 7.0 Hz, 1H), 7.11 (t, J = 6.8 Hz, 1H), 7.11 (t, J = 6.8 Hz, 1H), 7.06 (d, J = 10.1 Hz, 1H), 6.84 (t, J = 7.4 Hz, 1H), 6.54 (t, J = 8.2 Hz, 2H), 5.93 (d, J = 10.0 Hz, 1H), 5.70 (d, J = 7.5 Hz 1H), 3.01 (s, 3H).13C-NMR (151 MHz, DMSO-d6): δ = 152.2, 134.4, 130.4, 130.1, 128.5, 127.4, 127.1, 127.1, 126.7, 124.8, 123.5, 121.6, 120.1, 118.4, 113.6, 40.4. HRMS ESI-TOF+: m/z calcd for C18H16NO$^+$ [M+H$^+$]: 262.1226; found 262.1235.

***N*-([1,1'-biphenyl]-2-yl)acetamide** (**3**): Lithium hydroxide (727 mg, 17.3 mmol) was added to a solution of 2-aminobiphenyl (1.00 g, 5.91 mmol) in DCM (88 mL). The suspension was cooled to 0 °C before a solution of acetyl bromide (1.31 ml, 17.7 mmol) in DCM (8 mL) was added dropwise. The reaction mixture was stirred for 4 h at rt, filtered and concentrated under reduced pressure. The residue was dissolved in DCM and washed with water. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by crystallization at 0 °C from a DCM:*n*-heptane solution giving the product as colourless needles in 93 % yield (1.16 g, 5.48 mmol). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.27 (d, J = 8.1 Hz, 1H), 7.35 – 7.51 (m, 6H), 7.25 (d, J = 7.1 Hz, 1H), 7.18 (t, J = 7.3 Hz, 1H), 7.11 (s, 1H), 2.02 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.4, 138.3, 134.8, 132.3, 130.2, 129.4, 129.2, 128.6, 128.1, 124.5, 121.8, 24.8. LCMS: Calculated MH$^+$ (212); Found (MH$^+$) 212 m/z, Rt = 4.68 min.

6-methylphenanthridine (**4**): **3** (208 mg, 985 µmol) and polyphosphoric acid (3 ml, 115 % H_3PO_4 equiv.) were mixed and stirred for 2.5 h at 140 °C. The reaction was then cooled down to 0 °C and saturated aqueous sodium hydroxide solution was added until pH 14 was reached. The aqueous solution was then extracted with DCM and the organic phase was dried with sodium sulfate. Evaporation of the solvent under reduced pressure gave the product as a white solid in 87 % yield (165 mg, 854 mmol). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.64 (d, J = 8.3 Hz, 1H), 8.55 (d, J = 8.1 Hz, 1H), 8.23 (d, J = 8.2 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 7.85 (ddd, J = 8.3 Hz, 7.1, 1.3 Hz, 1H), 7.71 (m, 2H), 7.63 (ddd, J = 8.3, 7.1, 1.3 Hz, 1H), 3.06 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 159.0, 143.8, 132.7, 130.6, 129.5, 128.8, 127.5, 126.7, 126.5, 126.1, 123.9, 122.5, 122.1, 23.6. LCMS: Calculated MH$^+$ (194); Found (MH$^+$) 194 m/z, Rt = 3.91 min.

5,6-dimethylphenanthridin-5-ium trifluoromethanesulfonate (**5**): **4** (719 mg, 3.72 mmol) was dissolved in DCM (40 mL) at rt. Methyl trifluoromethanesulfonate (505 µL, 4.47 mmol) was then added dropwise and the reaction was left stirring at rt over night. The formed precipitate was filtered off, washed with Et_2O and dried under vacuum. The product was isolated in 89% yield (1.18 g, 3.30 mmol) as white needles. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (d, J = 8.0 Hz, 2H), 8.91 (d, J = 8.5 Hz, 1H), 8.63 (d, J = 8.3 Hz, 1H), 8.35 (t, J = 7.6 Hz, 1H), 8.16 – 8.01 (m, 3H), 4.57 (s, 3H), 3.43 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 165.9, 136.8, 135.0, 133.1, 131.7, 130.6, 130.2, 129.6, 124.5, 124.4, 124.4, 123.3, 120.7 ($J_{C\text{-}F}$ = 321 Hz), 120.0, 41.3, 19.6. LCMS: Calculated M^+ (cationic part only) (208); Found (M^+) 208 m/z, Rt = 0.95 min.

General procedure for preparation of spiropyrans. 2-hydroxybenzaldehyde (salicyl aldehyde) (1 equiv.) was dissolved in methanol (0.2 M) in a capped microwave vial at rt and piperidine (1 equiv.) was added. The mixture was then heated to reflux before it was removed from the oilbath and 0.25 equiv. of triflate salt of 5,6-dimethylphenanthridin-5-ium (**5**) in methanol (0.2 M) was added. The reaction was then refluxed for 20 min and then again removed from the oilbath and an additional 0.25 equiv. of **5** was added. This sequence was repeated until a total of 1 equiv. of **5** had been added. The reaction was then left to reflux for an additional hour and then allowed to cool down under stirring over night. Unless otherwise noted, the formed precipitate was filtered off over a small glass-frit funnel and then dried under vacuum to give the phenanthridine spiropyrans as racemates. No additional purification was needed.

5'-Methyl-5'H-spiro[chromene-2,6'-phenanthridine] (**6**) (ECH24): Made using the general procedure from **5** (53 mg, 0.15 mmol) and salicyl aldehyde (15 µL, 0.15 mmol), giving **6** as a white solid in 74% yield (34 mg, 0.11 mmol). ^{1}H NMR (600 MHz, DMSO-d6) δ 8.14 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 7.4 Hz, 1H), 7.41 – 7.34 (m, 3H), 7.27 (d, J = 7.5, 1H), 7.17 (d, J = 10.0 Hz, 1H), 7.10 – 7.01 (m, 3H), 6.86 (t, J = 7.4, 1H), 6.46 (d, J = 8.0 Hz, 1H), 6.02 (d, J = 10.0 Hz, 1H), 3.10 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 151.9, 140.8, 131.4, 130.1, 129.6, 129.0, 128.2, 127.8, 127.5, 127.2, 127.0, 123.1, 123.0, 122.1, 120.4, 119.6, 119. 2, 118.4, 114.9, 114.0, 91.2, 33.0. HRMS ESI-TOF+: m/z calcd for C22H18NO$^+$ [M+H$^+$]: 312.1383; found 312.1393.

8-Methoxy-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**7**) (ECH25): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 2-hydroxy-3-

methoxybenzaldehyde (18 µL, 0.14 mmol), giving **7** as a white solid in 69% yield (33 mg, 0.10 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.15 (d, *J* = 7.9, 1H), 8.11 (d, *J* = 7.9, 1H), 7.49 (td, *J* = 7.3, 1.9 Hz, 1H), 7.41 – 7.34 (m, 3H), 7.13 (d, *J* = 10.0 Hz, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 7.02 (t, *J* = 7.3 Hz, 2H), 6.88 (dd, *J* = 7.0, 2.2 Hz, 1H), 6.84 – 6.78 (m, 2H), 5.98 (d, *J* = 10.0 Hz, 1H), 3.46 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 146.6, 141.0, 140.6, 131.2, 129.6, 129.0, 128.1, 127.5, 126.8, 123.1, 123.0, 122.0, 119.9, 119.4, 119.3, 119.1, 118.7, 113.8, 113.7, 91.2, 55.4, 32.9. HRMS ESI-TOF+: *m/z* calcd for C23H20NO2$^+$ [M+H$^+$]: 342.1489; found 342.1498.

7-Methoxy-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**8**) (ECH26): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 2-hydroxy-4-methoxybenzaldehyde (14 µL, 0.14 mmol), giving **8** as a white solid in 71% yield (34 mg, 0.10 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.17 – 8.13 (d, *J* = 8.1 Hz, 1H), 8.10 (d, *J* = 8.1 Hz, 1H), 7.50 (t, *J* = 7.3 Hz, 1H), 7.44 – 7.34 (m, 3H), 7.17 (d, *J* = 8.4 Hz, 1H), 7.11 (d, *J* = 10.0 Hz, 1H), 7.08 (d, *J* = 8.1 Hz, 1H), 7.06 – 7.02 (t, *J* = 7.4 Hz, 1H), 6.43 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.08 (d, *J* = 2.4 Hz, 1H), 5.84 (d, *J* = 10.0 Hz, 1H), 3.61 (s, 3H), 3.11 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 161.1, 153.3, 140.8, 131.4, 129.5, 129.0, 128.2, 128.0, 127.9, 127.5, 126.7, 123.0, 122.0, 120.1, 119.6, 119.2, 114.0, 111.5, 107.0, 100.1, 91.5, 55.1, 33.0. HRMS ESI-TOF+: *m/z* calcd for C23H20NO2$^+$ [M+H$^+$]: 342.1489; found 342.1499.

6-Methoxy-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**9**) (ECH27): Made using the general procedure from **5** (20 mg, 0.06 mmol) and 2-hydroxy-5-methoxybenzaldehyde (7 µL, 0.06 mmol), giving **9** as a white solid in 52% yield (10 mg, 0.03 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (d, *J* = 7.9 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.48 (t, *J* = 6.7 Hz, 1H), 7.38 – 7.34 (m, *J* = 2.0 Hz, 2H), 7.14 (d, *J* = 10.0 Hz, 1H), 7.07 – 7.00 (m, 2H), 6.89 (d, *J* = 3.0 Hz, 1H), 6.65 (dd, *J* = 8.7, 3.0 Hz, 1H), 6.40 (d, *J* = 8.7 Hz, 1H), 6.04 (d, *J* = 10.0 Hz, 1H), 3.70 (s, 3H), 3.08 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 153.0, 145.8, 140.9, 131.4, 129.5, 129.0, 128.2, 127.7, 127.4, 127.1, 123.9, 123.0, 122.1, 119.6, 119.1, 118.9, 115.7, 115.6, 113.9, 111.7, 90.8, 55.4, 33.1. HRMS ESI-TOF+: *m/z* calcd for C23H20NO2$^+$ [M+H$^+$]: 342.1489; found 342.1496.

5-Methoxy-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**10**) (ECH28): Made using the general procedure from **5** (30 mg, 0.08 mmol) and methyl 2-hydroxy-6-methoxybenzaldehyde (13 mg, 0.08 mmol), giving **10** as a white solid in 52% yield (15 mg, 0.04 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (dd, *J* = 8.0, 7.7 Hz, 2H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.44 – 7.29 (m, 4H), 7.06 – 7.01 (m, 3H), 6.52 (d, *J* = 8.3 Hz, 1H), 6.09 (d, *J* = 8.2 Hz, 1H), 5.93 (d, *J* = 10.2 Hz, 1H), 3.86 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 155.2, 152.5, 140.7, 131.4, 130.3, 129.6, 129.0, 128.2, 127.9, 127.5, 123.0, 122.1, 121.4, 121.0, 119.6, 119.2, 114.0, 107.9, 107.2, 102.9, 90.9, 55.7, 32.9. HRMS ESI-TOF+: *m/z* calcd for C23H20NO2$^+$ [M+H$^+$]: 342.1489; found 342.1497.

5',8-Dimethyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**11**) (ECH29): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 2-hydroxy-3-methylbenzaldehyde (17 µL, 0.14 mmol), giving **11** as a white solid in 70% yield (32 mg, 0.10 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (dd, *J* = 8.0, 1.2 Hz, 1H), 8.09 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.49 (ddd, *J* = 7.9, 7.1, 1.5 Hz, 1H), 7.41 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.36 (dddd, *J* = 8.0, 7.0, 4.2, 1.4 Hz, 2H), 7.14 (d, *J* = 10.1 Hz, 1H), 7.10 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.06 – 7.00 (m, 2H), 6.96 (ddd, *J* = 7.5, 1.8, 0.9 Hz, 1H), 6.77 (t, *J* = 7.5 Hz, 1H), 6.04 (d, *J* = 10.0 Hz, 1H), 3.03 (s, 3H), 1.66 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 149.8, 140.9, 132.1, 131.2, 129.5, 128.9, 128.5, 127.6, 127.3, 127.3, 124.8, 123.5, 123.0, 122.7, 122.1, 119.9, 119.2, 118.2, 113.9, 90.6, 32.7, 14.7. HRMS ESI-TOF+: *m/z* calcd for C23H20NO$^+$ [M+H$^+$]: 326.1539; found 326.1548.

5',6-Dimethyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**12**) (ECH30): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 2-hydroxy-5-methylbenzaldehyde (19 mg, 0.14 mmol), giving **12** as a white solid in 77% yield (35 mg, 0.11 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (d, *J* = 7.9 Hz, 1H), 8.09 (d, *J* = 7.9 Hz, 1H), 7.49 (t, *J* = 7.4 Hz, 1H), 7.41 – 7.34 (m, 3H), 7.12 (d, *J* = 10.0 Hz, 1H), 7.08 – 7.02 (m, 3H), 6.89 – 6.86 (d, *J* = 8.4 Hz, 1H), 6.36 (d, *J* = 8.2 Hz, 1H), 6.00 (d, *J* = 10.0 Hz, 1H), 3.09 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 149.8, 140.8, 131.5, 130.5, 129.6, 129.1, 129.0, 128.2, 127.8, 127.5, 127.1, 123.2, 123.0, 122.1, 119.6, 119.2, 118.2, 114.7, 113.9, 91.0, 33.0, 20.1. HRMS ESI-TOF+: *m/z* calcd for C23H20NO$^+$ [M+H$^+$]: 326.1539; found 326.1548.

5-Bromo-8-methoxy-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**13**) (ECH31): Made using the general procedure from **5** (30 mg, 0.08 mmol) and **o** (19 mg, 0.08 mmol), giving **13** as a white solid in 74% yield (26 mg, 0.06 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.17 (d, *J* = 8.1 Hz, 1H), 8.12 (d, *J* = 7.9 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.43 – 7.36 (m, 2H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 10.2 Hz, 1H), 7.11 – 7.03 (m, 3H), 6.80 (d, *J* = 8.8 Hz, 1H), 6.17 (d, *J* = 10.2 Hz, 1H), 3.47 (s, 3H), 3.08 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 146.6, 142.4, 140.3, 130.5, 129.7, 129.3, 128.1, 128.1, 127.7, 125.2, 125.0, 123.2, 123.1, 122.1, 119.4, 119.2, 117.6, 114.6, 113.9, 111.7, 91.8, 55.6, 33.0. HRMS ESI-TOF+: *m/z* calcd for C23H19BrNO2$^+$ [M+H$^+$]: 420.0594; found 420.0598.

5'-Methyl-5'*H*-spiro[chromene-2,6'-phenanthridin]-8-ol (**14**) (ECH32): Made using the general procedure from **5** (20 mg, 0.06 mmol) and 2,3-dihydroxybenzaldehyde (8.0 mg, 0.06 mmol). The crude mixture was concentrated and purified by preparative HPLC using MeCN:water with 0.75% formic acid as eluent, giving the open zwitterionic intermediate after condensation with the aldehyde. The isolated intermediate was then dissolved in a small amount of methanol and poured into 5% KOH in water. The closed spirocyclic compound was then extracted by EtOAc and dried under vacuum giving **14** in 66% yield (12 mg, 0.04 mmol). Trace amounts of ring-opened merocyanine form still present. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, *J* = 7.9 Hz, 1H), 8.09 (d, *J* = 7.9 Hz, 1H), 7.48 (t, *J* = 7.4 Hz, 1H), 7.41 – 7.32 (m, 3H), 7.10 – 6.98 (m, 3H), 6.74 – 6.61 (m, 3H), 5.92 (d, *J* = 10.0 Hz, 1H), 3.07 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 144.3, 140.8, 139.9, 131.5, 129.5, 128.9, 128.2, 127.4, 126.9, 123.0, 122.9, 121.8, 119.9, 119.2, 119.0, 118.9, 117.7, 117.1, 113.7, 90.7, 32.9. HRMS ESI-TOF+: *m/z* calcd for C22H18NO2$^+$ [M+H$^+$]: 328.1332; found 328.1338.

8-Ethyl-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**15**) (ECH33): Made using the general procedure from **5** (20 mg, 0.06 mmol) and 3-ethyl-2-hydroxybenzaldehyde (8.0 mg, 0.06 mmol), giving **15** as a white solid in 87% yield (17 mg, 0.05 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (d, *J* = 8.0 Hz, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.43 – 7.33 (m, 3H), 7.15 (d, *J* = 10.0 Hz, 1H), 7.10 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.06 – 7.00 (m, 2H), 6.95 (dd, *J* = 7.5 Hz, 1H), 6.78 (t, *J* = 7.5 Hz, 1H), 6.04 (d, *J* = 10.0 Hz, 1H), 3.06 (s, 3H), 2.10 – 2.02 (m, 2H), 0.65 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 149.5, 140.9, 131.9, 129.9, 129.7, 129.4, 128.9, 128.5, 127.6, 127.4, 127.3, 124.9, 122.9, 122.7, 122.1, 120.1, 119.9, 119.2, 118.3, 113.9, 90.6, 32.9, 22.3, 13.7. HRMS ESI-TOF+: *m/z* calcd for C24H22NO$^+$ [M+H$^+$]: 340.1696; found 340.1703.

6,8-Dibromo-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**16**) (ECH34): Made using the general procedure from **5** (30 mg, 0.08 mmol) and 3,5-dibromosalicylaldehyde (23 mg, 0.08 mmol), giving **16** as a white solid in 61% yield (24 mg, 0.05 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.19 (d, *J* = 7.9 Hz, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 7.57 (dd, *J* = 8.5, 2.3 Hz, 2H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.43 – 7.34 (m, 3H), 7.19 (d, *J* = 10.1 Hz, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 6.21 (d, *J* = 10.1 Hz, 1H), 3.07 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 148.0, 140.2, 134.4, 130.5, 129.6, 129.4, 129.0, 128.4, 127.8, 127.6, 125.8, 124.9, 123.1, 122.2, 121.8, 119.6, 119.5, 114.0, 111.4, 109.6, 93.3, 32.9. HRMS ESI-TOF+: *m/z* calcd for C22H16Br2NO$^+$ [M+H$^+$]: 467.9593; found 467.9597.

6,8-Dichloro-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**17**) (ECH35): Made using the general procedure from **5** (30 mg, 0.08 mmol) and 3,5-dichloro-2-hydroxybenzaldehyde (16 mg, 0.08 mmol), giving **17** as a white solid in 85% yield (27 mg, 0.07 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.20 (d, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 7.54 (t, *J* = 7.3 Hz, 1H), 7.44 (d, *J* = 2.5 Hz, 1H), 7.43 – 7.34 (m, 4H), 7.22 (d, *J* = 10.1 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.24 (d, *J* = 10.1 Hz, 1H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 148.0, 140.2, 134.4, 130.5, 129.7, 129.4, 129.0, 128.4, 127.8, 127.6, 125.8, 124.9, 123.1, 122.2, 121.8, 119.6, 119.5, 114.0, 111.4, 109.6, 93.3, 32.9. HRMS ESI-TOF+: *m/z* calcd for C22H16Cl2NO$^+$ [M+H$^+$]: 380.0603; found 380.0611.

6-Fluoro-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**18**) (ECH36): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 5-fluorosalicylaldehyde (20 mg, 0.14 mmol), giving **18** as a white solid in 76% yield (35 mg, 0.11 mmol).^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 7.9 Hz, 1H), 7.50 (m, 1H), 7.41 – 7.34 (m, 2H), 7.21 – 7.14 (m, 2H), 7.07 (d, *J* = 8.4 Hz 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.89 (td, *J* = 8.4, 3.0 Hz, 1H), 6.48 (dd, *J* = 8.7, 4.5 Hz, 1H), 6.12 (d, *J* = 10.0 Hz, 1H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 156.8, 155.3, 148.1, 140.7, 131.1, 129.6, 129.1, 128.2, 127.7, 127.6, 126.4, 124.5, 123.1, 122.2, 119.6, 119.5, 119.4, 119.3, 116.3, 116.1, 116.1, 114.0, 113.2, 113.0, 91.4, 33.1. HRMS ESI-TOF+: *m/z* calcd for C22H17FNO$^+$ [M+H$^+$]: 330.1289; found 330.1294.

6-Bromo-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**19**) (ECH37): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 5-bromosalicylaldehyde (28 mg, 0.14 mmol), giving **19** as a white solid in 71% yield (39 mg, 0.10 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, *J* = 8.0 Hz, 1H), 8.10 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.55 – 7.47 (m, 2H), 7.41 – 7.34 (m, 3H), 7.23 – 7.15 (m, 2H), 7.11 – 7.02 (m, 2H), 6.45 (d, *J* = 8.6 Hz, 1H), 6.11 (d, *J* = 10.1 Hz, 1H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 151.1, 140.5, 132.3, 130.9, 129.7, 129.4, 129.2, 128.2, 127.8, 127.6, 126.0, 124.3, 123.1, 122.2, 120.7, 119.5, 119.4, 117.2, 114.0, 111.4, 91.8, 33.1. HRMS ESI-TOF+: *m/z* calcd for C22H17BrNO$^+$ [M+H$^+$]: 390.0488; found 390.0497.

6-(*tert*-Butyl)-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**20**) (ECH38): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 5-*tert*-butyl-2-hydroxybenzaldehyde (24 µL, 0.14 mmol), giving **20** as a white solid in 72% yield (37 mg, 0.10 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, *J* = 7.9 Hz, 1H), 8.09 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.48 (dt, *J* = 7.4, 2.3 Hz, 1H), 7.41 – 7.33 (m, 3H), 7.28 (d, *J* = 2.4 Hz, 1H), 7.17 (d, *J* = 10.0 Hz, 1H), 7.12 – 6.99 (m, 3H), 6.39 (d, *J* = 8.5 Hz, 1H), 5.98 (d, *J* = 10.0 Hz, 1H), 3.08 (s, 3H), 1.25 (s, 9H). ^{13}C NMR (151 MHz, DMSO) δ 149.7, 142.5, 140.8, 131.6, 129.6, 129.0, 128.2, 127.8, 127.5, 127.4, 127.0, 123.9, 123.0, 122.8, 122.1, 119.6, 119.2, 117.5, 114.2, 113.9, 91.0, 33.8, 33.0, 31.3. HRMS ESI-TOF+: *m/z* calcd for C26H26NO$^+$ [M+H$^+$]: 368.2009; found 368.2018.

5'-Methyl-6-phenyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**21**) (ECH39): Made using the general procedure from **5** (20 mg, 0.06 mmol) and **29** (11 mg, 0.06 mmol), giving **21** as a white solid in 78% yield (17 mg, 0.04 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.17 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 7.8 Hz, 1H), 7.66 – 7.59 (m, 4H), 7.53 (t, *J* = 7.5 Hz, 1H), 7.47 – 7.35 (m, 5H), 7.32 (t, *J* = 7.3 Hz, 1H), 7.27 (d, *J* = 10.1 Hz, 1H), 7.10 (d, *J* = 8.2 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 6.57 (d, *J* = 8.2 Hz, 1H), 6.09 (d, *J* = 10.1 Hz, 1H), 3.14 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 151.6, 140.7, 139.6, 132.4, 131.3, 129.6, 129.1, 128.9, 128.3, 128.2, 127.8, 127.6, 127.1, 126.8, 126.1, 125.5, 123.5, 123.1, 122.1, 119.6, 119.3, 118.7, 115.4, 114.0, 91.6, 33.0. HRMS ESI-TOF+: *m/z* calcd for C28H22NO$^+$ [M+H$^+$]: 388.1696; found 388.1708.

***N*,*N*-diethyl-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridin]-6-amine** (**22**) (ECH40): Prepared using the general procedure from **5** (30 mg, 0.08 mmol) and 4-diethylaminosalicylaldehyde (16 mg, 0.08 mmol). The crude mixture was concentrated and purified by preparative HPLC using MeCN:water with 0.75% formic acid as eluent, giving the open zwitterionic intermediate after condensation with the aldehyde, as a deep purple solid. The isolated solid was then dissolved in a small amount of methanol and poured into 5% KOH in water. The closed spirocyclic compound was then extracted by EtOAc and dried under vacuum giving **22** in 11% yield (3.5 mg, 0.0092 mmol). Trace amounts of ring-opened merocyanine form still present. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 – 8.06 (dd, *J* = 8.2, 7.9 Hz, 2H), 7.50 – 7.40 (m, 2H), 7.38 – 7.33 (m, 2H), 7.08 – 6.96 (m, 4H), 6.16 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.68 (d, *J* = 2.5 Hz, 1H), 5.62 (d, *J* = 9.9 Hz, 1H), 3.19 (q, *J* = 7.0 Hz, 4H), 3.10 (s, 3H), 0.96 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO) δ 153.5, 149.4, 141.0, 131.9, 129.5, 128.8, 128.2, 128.1, 128.0, 127.4, 126.8, 123.0, 121.9, 119.5, 118.9, 117.5,

113.9, 106.6, 103.9, 96.8, 91.1, 43.5, 32.9, 12.5. HRMS ESI-TOF+: *m/z* calcd for $C_{26}H_{27}N_2O^+$ [M+H$^+$]: 383.2118; found 383.2128.

Methyl 5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine]-6-carboxylate (**23**) (ECH41): Made using the general procedure from **5** (50 mg, 0.14 mmol) and methyl 3-formyl-4-hydroxybenzoate (21 µL, 0.14 mmol), giving **23** as a white solid in 77% yield (40 mg, 0.11 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 7.95 (d, *J* = 2.2 Hz, 1H), 7.67 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.54 – 7.50 (m, 1H), 7.41 – 7.36 (m, 3H), 7.32 (d, *J* = 10.2 Hz, 1H), 7.12 – 7.03 (m, 2H), 6.57 (d, *J* = 8.5 Hz, 1H), 6.14 (d, *J* = 10.2 Hz, 1H), 3.81 (s, 3H), 3.11 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 165.7, 155.9, 140.4, 131.5, 130.8, 129.7, 129.3, 128.8, 128.2, 127.8, 127.7, 126.6, 123.8, 123.1, 122.2, 121.6, 119.6, 119.5, 118.4, 115.3, 114.1, 92.7, 51.9, 33.0. HRMS ESI-TOF+: *m/z* calcd for $C_{24}H_{20}NO_3^+$ [M+H$^+$]: 370.1438; found 370.1447.

6-Iodo-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**24**) (ECH42): Made using the general procedure from **5** (20 mg, 0.06 mmol) and 5-iodosalicylaldehyde (14 mg, 0.06 mmol), giving **24** as a white solid in 70% yield (17 mg, 0.04 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 7.9, 1H), 7.65 (d, *J* = 2.2 Hz, 1H), 7.52 – 7.48 (m, 1H), 7.43 – 7.31 (m, 4H), 7.16 (d, *J* = 10.1 Hz, 1H), 7.10 – 7.01 (m, 2H), 6.32 (d, *J* = 8.5 Hz, 1H), 6.08 (d, *J* = 10.1 Hz, 1H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 151.8, 140.5, 138.2, 135.2, 131.0, 129.6, 129.2, 128.2, 127.8, 127.6, 125.9, 124.0, 123.1, 122.1, 121.2, 119.5, 119.4, 117.6, 114.0, 91.7, 82.5, 33.0. HRMS ESI-TOF+: *m/z* calcd for $C_{22}H_{17}INO^+$ [M+H$^+$]: 438.0349; found 438.0350.

5'-Methyl-5'*H*-spiro[chromene-2,6'-phenanthridine]-6-carboxylic acid (**25**) (ECH43): Made using the general procedure from **5** (50 mg, 0.14 mmol) and **30** (23 mg, 0.14 mmol). Removal of unknown byproduct was accomplished by several recrystalisations using DCM and MeOH, finally giving **25** as a beige solid in 30% yield (15 mg, 0.04 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, *J* = 7.9 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 7.91 (d, *J* = 2.2 Hz, 1H), 7.65 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.54 – 7.50 (m, 1H), 7.42 – 7.38 (m, 3H), 7.30 (d, *J* = 10.1 Hz, 1H), 7.11 – 7.05 (m, 2H), 6.54 (d, *J* = 8.5 Hz, 1H), 6.12 (d, *J* = 10.1 Hz, 1H), 3.12 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 166.8, 155.6, 140.5, 131.6, 130.9, 129.7, 129.3, 129.0, 128.2, 127.9, 127.7, 126.7, 123.6, 123.1, 122.2, 119.5, 118.1, 115.0, 114.1, 92.5, 33.0. HRMS ESI-TOF+: *m/z* calcd for $C_{23}H_{18}NO_3^+$ [M+H$^+$]: 356.1281; found 356.1294.

5-Methyl-5*H*-spiro[phenanthridine-6,2'-pyrano[2,3-*b*]pyridine] (**26**) (ECH44): Made using the general procedure from **5** (35 mg, 0.10 mmol) and 2-oxo-1,2-dihydro-3-pyridinecarbaldehyde (12 mg, 0.10 mmol), giving the open cationic intermediate after condensation with the aldehyde, as a beige solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (bs, 1H), 9.17 (d, *J* = 7.8 Hz, 2H), 8.72 – 8.51 (m, 3H), 8.36 (t, *J* = 7.7 Hz, 1H), 8.23 – 7.90 (m, 3H), 7.66 (d, *J* = 5.6 Hz, 1H), 7.26 (d, *J* = 16.2 Hz, 1H), 6.45 (t, *J* = 6.5 Hz, 1H), 4.57 (s, 3H).

The isolated solid was then dissolved in a small amount of methanol and poured into 5% KOH in water. The closed spirocyclic compound was then extracted by EtOAc and dried under vacuum giving **26** in 52% yield (16 mg, 0.05 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, *J* = 7.9 Hz, 1H), 8.13 (d, *J* = 7.7 Hz, 1H), 7.91 (dd, *J* = 5.0, 2.0 Hz, 1H), 7.71 (dd, *J* = 7.3, 2.0 Hz, 1H), 7.55 – 7.51 (m, 1H), 7.44 – 7.34 (m, 3H), 7.22 (d, *J* = 10.0 Hz, 1H), 7.14 – 7.07 (m, 2H), 6.93 (dd, *J* = 7.3, 5.0 Hz, 1H), 6.16 (d, *J* = 10.0 Hz, 1H), 3.11 (s, 3H). HRMS ESI-TOF+: *m/z* calcd for $C_{21}H_{17}N_2O$ [M+H$^+$]: 313.1335; found 313.1339.

5'-Methyl-5'*H*-spiro[benzo[*f*]chromene-3,6'-phenanthridine] (**27**) (ECH45): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 2-hydroxy-1-naphtaldehyde (24 mg, 0.14 mmol), giving **27** as a gray solid in 81% yield (41 mg, 0.11 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, *J* = 8.5 Hz, 1H), 8.17 (d, *J* = 8.1 Hz, 1H), 8.13 (d, *J* = 7.5 Hz, 1H), 7.98 (d, *J* = 10.4 Hz, 1H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.69 (d, *J* = 8.9 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.54 – 7.43 (m, 2H), 7.42 – 7.33 (m, 3H), 7.11 – 7.03 (m, 2H), 6.77 (d, *J* = 8.9 Hz, 1H), 6.13 (d, *J* = 10.4 Hz, 1H), 3.13 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 150.0, 140.6, 131.4, 130.4, 129.6, 129.6, 129.1, 128.4, 128.4, 128.2, 127.6, 127.0, 123.5, 123.1, 122.7, 122.0, 121.6, 121.2, 119.6, 119.3, 117.3, 114.0, 110.1, 91.3, 32.9. HRMS ESI-TOF+: *m/z* calcd for $C_{26}H_{20}NO^+$ [M+H$^+$]: 362.1539; found 362.1550.

***N*-(3,4-dimethoxyphenyl)-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine]-6-carboxamide** (**28**) (ECH46): Made using the general procedure from **5** (35 mg, 0.10 mmol) and **31** (42 mg, 0.10 mmol), giving **28** as a gray solid in 56% yield (27 mg, 0.06 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 7.92 (d, *J* = 2.3 Hz, 1H), 7.71 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.46 – 7.37 (m, 4H), 7.34 – 7.26 (m, 2H), 7.11 (d, *J* = 8.3 Hz, 1H), 7.09 – 7.05 (t, *J* = 7.4 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 6.15 (d, *J* = 10.1 Hz, 1H), 3.75 (s, 3H), 3.73 (s, 3H), 3.13 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 164.3, 154.5, 148.4, 145.0, 140.5, 132.9, 131.0, 129.7, 129.3, 128.2, 127.8, 127.6, 127.1, 127.1, 126.8, 123.7, 123.1, 122.2, 119.6, 119.5, 118.0, 114.7, 114.1, 112.1, 111.9, 105.4, 92.3, 55.7, 55.4, 33.1. HRMS ESI-TOF+: *m/z* calcd for $C_{31}H_{26}N_2O_4^+$ [M+H$^+$]: 491.1965; found 491.1968.

Folding of the G4 structures

The oligonucleotides used in this study were purchased from Eurofins MWG Operon (Germany). To fold the oligonucleotides, 50 µM oligonucleotide solution was prepared in 10 mM Tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl; pH 7.5) and 100 mM KCl, heated to 95 °C for 5 min, and slowly cooled to room temperature overnight.

Thioflavin T (ThT) displacement assay

1 µM folded oligonucleotides was incubated in 0.5 µM ThT, 10 mM Tris pH=7.5, 100 mM KCl treated with 0.0002, 0.0008, 0.003, 0.01, 0.25, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.13, 6.25, 12.5, 25 or 50 µM concentration of the compounds in a final volume of 40 µl. 2.5% v/v DMSO was used as a reference sample. All compounds were dissolved in 100% v/v DMSO. The final concentration of DMSO in each sample was 2.5%. 10 µL from the mixture was transferred into three wells in 384-well Corning black flat-bottom microplates to obtain technical triplicates. BioTek Synergy H4 Microtiter plate reader was used for readout at λ = 435/480 nm (ex/em). Background

fluorescence of the buffer containing appropriate compound was subtracted from all the measurements. The relative fluorescence was calculated as a fold change (increase or decrease) to reference sample treated with DMSO. DC_{50} values of ThT displacement were calculated using Origin 8.5 software by fitting a dose response function with fixed bottom asymptote to 0.

CD measurements

For CD measurements, 50 μM folded oligonucleotide were diluted to 5 μM concentration in a buffer containing 10 mm Tris–HCl (pH 7.5) and 100 mm KCl and in the presence or absence of 80 μM of the tested compound. The blank sample contained the same buffer without the compound but with 1.25% (v/v) DMSO. A JASCO-720 spectropolarimeter with Peltier temperature control was used for the measurements. Four accumulations of CD spectra of 5 μM oligonucleotide were recorded at 25 °C over λ = 230–350 nm. The measurements were performed in a quartz cuvette with a path length of 0.1 cm. For melting curves, the wavelength was held constant at λ =264 nm and melting curves were recorded in a range of 25-90 °C. The T_m value is defined as the temperature at which 50% of the G4 structures are unfolded. T_m values were estimated by fitting the melting curves into a dose response function in Origin 8.5 Software.

Taq-polymerase stop assay

1 μM TET-labeled primer was annealed to 1.25 μM template DNA in 100 mM KCl by heating at 95 °C for 5 min and was slowly cooled to room temperature, overnight. Annealed DNA (40 nm) was incubated with 25 μM compound or 5% (v/v) DMSO for 30 min in 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 1.5 mM $MgCl_2$, and 200 mM dNTPs (dATP, dTTP, dCTP, dGTP) at room temperature. Primer extension assay was accomplished by the addition of 1 U of Taq DNA polymerase (Thermo Scientific) diluted in 10 mM Tris-HCl (pH 7.5) and 50 mM KCl into the reaction mixture at 50 °C. The final volume of the reaction was 45 μL. Reaction was stopped after 0.5, 1, 5 or 10 minutes by transferring 10 uL of the reaction mixture into a stop solution (containing 95% formamide, 20 mM ethylenediaminetetraacetic acid (EDTA), and 0.1% bromophenol blue). 5 uL of the final mixture was loaded onto a 10% polyacrylamide gel containing 8 M urea, 25% formamide and 1×Tris/borate/ethylenediaminetetraacetic acid (TBE). The gel was visualized with a Typhoon Scanner 9400 (GE Healthcare) and quantified with the Image Quant 5.2 software (GE Healthcare).

NMR Analysis

The reference sample of Pu24T was prepared by adding 20mL D_2O to 200mL stock solution of Pu24T (100 mM Pu24T in PBS buffer 10mM phosphate, 25mM KCl, pH 7) and transferring the solution to a 3mm NMR tube.

Samples containing compound 7 or 8 were prepared in the same way as the reference sample but with addition of 2mL of stock solution of 7 or 8 (10mM in DMSO) to get a 1:1 ratio between Pu24T and the added compound.

All spectra were acquired on a Bruker 850MHz Avance III HD spectrometer equipped with a 5mm TCI cryo-probe. 256 scans were added with a relaxation delay of 1s and with excitation sculpting for water suppression. 1Hz line-broadening was applied before fourier transform. All processing was done in Topspin 3.2 (www.bruker.com).

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Notes and references

1 (a) M. Gellert, M. N. Lipsett and D. R. Davies, *Proc. Natl. Acad. Sci. USA*, 1962, **48**, 2013;(b) D. Sen and W. Gilbert, *Nature*, 1988, **334**, 364.

2 (a) F. B. Howard and H. T. Miles, *Biochemistry*, 1982, **21**, 6736;(b) J. R. Williamson, M. K. Raghuraman and T. R. Cech, *Cell*, 1989, **59**, 871.

3 (a) A. Guedin, J. Gros, P. Alberti and J.-L. Mergny, *Nucleic Acids Res.*, 2010, **38**, 7858;(b) S. Burge, G. N. Parkinson, P. Hazel, A. K. Todd and S. Neidle, *Nucleic Acids Res.*, 2006, **34**, 5402;(c) P. Hazel, G. N. Parkinson and S. Neidle, *Nucleic Acids Res.*, 2006, **34**, 2117;(d) S. Neidle, *Curr. Opin. Struct. Biol.*, 2009, **19**, 239.

4 (a) N. Maizels and L. T. Gray, *PLoS Genet.*, 2013, **9**, 6;(b) A. Siddiqui-Jain, C. L. Grand, D. J. Bearss and L. H. Hurley, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 11593;(c) B. Maji and S. Bhattacharya, *Chem. Commun.*, 2014, **50**, 6422;(d) A. M. Zahler, J. R. Williamson, T. R. Cech and D. M. Prescott, *Nature*, 1991, **350**, 718.

5 (a) S. Balasubramanian, L. H. Hurley and S. Neidle, *Nat. Rev. Drug Discov.*, 2011, **10**, 261;(b) S. Neidle, *J. Med. Chem.*, 2016, **59**, 5987;(c) Y. L. Wu and R. M. Brosh, *FEBS J.*, 2010, **277**, 3470;(d) N. Maizels, *EMBO Rep.*, 2015, **16**, 910.

6 (a) Z. A. E. Waller, B. J. Pinchbeck, B. S. Buguth, T. G. Meadows, D. J. Richardson and A. J. Gates, *Chem. Commun.*, 2016, **52**, 13511;(b) K. Tsukakoshi, Y. Ikuta, K. Abe, W. Yoshida, K. Iida, Y. Ma, K. Nagasawa, K. Sode and K. Ikebukuro, *Chem. Commun.*, 2016, **52**, 12646;(c) D. Drygin, A. Siddiqui-Jain, S. O'Brien, M. Schwaebe, A. Lin, J. Bliesath, C. B. Ho, C. Proffitt, K. Trent, J. P. Whitten, J. K. C. Lim, D. Von Hoff, K. Anderes and W. G. Rice, *Cancer Res.*, 2009, **69**, 7653;(d) X. X. Huang, L. N. Zhu, B. Wu, Y. F. Huo, N. N. Duan and D. M. Kong, *Nucleic Acids Res.*, 2014, **42**, 8719;(e) L. N. Zhu, B. Wu and D. M. Kong, *Nucleic Acids Res.*, 2013, **41**, 4324.

7 J. Jamroskovic, M. Livendahl, J. Eriksson, E. Chorell and N. Sabouri, *Chem. Eur. J.*, 2016, **22**, 18932.

8 (a) L. K. Smith and I. R. Baxendale, *Org. Biomol. Chem.*, 2015, **13**, 9907;(b) Y. J. Zheng, C. M. Tice and S. B. Singh, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 3673.

9 F. Lovering, J. Bikker and C. Humblet, *J. Med. Chem.*, 2009, **52**, 6752.

10 (a) D. Monchaud and M. P. Teulade-Fichou, *Org. Biomol. Chem.*, 2008, **6**, 627;(b) T. M. Ou, Y. J. Lu, J. H. Tan, Z. S. Huang, K. Y. Wong and L. Q. Gu, *ChemMedChem*, 2008, **3**, 690.

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DOI: 10.1039/C7OB00300E

11 E. R. Zakhs, L. A. Zvenigorodskaya, N. G. Leshenyuk and V. P. Martynova, *Chem. Heterocycl. Compd.*, 1977, **13**, 1055.
12 M. Livendahl, J. Jamroskovic, S. Ivanova, P. Demirel, N. Sabouri and E. Chorell, *Chem. Eur. J.*, 2016, **22**, 13004.
13 M. Wallgren, J. B. Mohammad, K. P. Yan, P. Pourbozorgi-Langroudi, M. Ebrahimi and N. Sabouri, *Nucleic Acids Res.*, 2016, **44**, 6213.
14 A. T. Phan, V. Kuryavyi, H. Y. Gaw and D. J. Patel, *Nat. Chem. Biol.*, 2005, **1**, 167.
15 A. Marchand, A. Granzhan, K. Iida, Y. Tsushima, Y. Ma, K. Nagasawa, M.-P. Teulade-Fichou and V. Gabelica, *J. Am. Chem. Soc.*, 2015, **137**, 750.
16 W. J. Chung, B. Heddi, F. Hamon, M.-P. Teulade-Fichou and P. Anh Tuan, *Angew. Chem. Int. Ed.*, 2014, **53**, 999.
17 M. Zaidlewicz, A. Tafelska-Kauzmarek, A. Prewysz-Kwinto and A. Chechlowska, *Tetrahedron-Asymmetry*, 2003, **14**, 1659.

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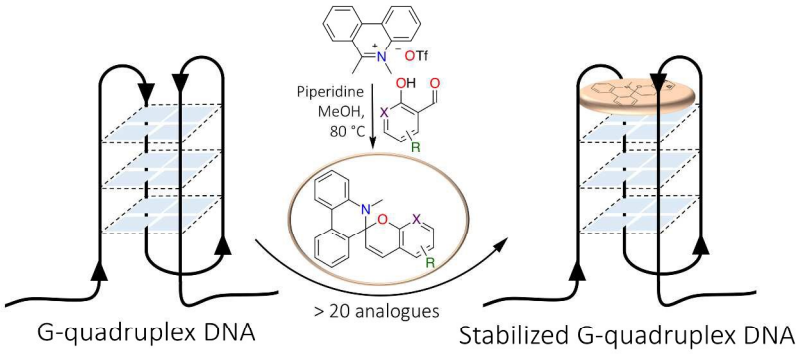
Low molecular weight spirocycles efficiently stabilize G-quadruplex DNA without changing its structure by binding the top of the G-quadruplex structure.



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