Nitrogen Heterocycles

The Relation Between Position and Chemical Composition of Bis-Indole Substituents Determines Their Interactions with G-Quadruplex DNA

Bagineni Prasad,[a] Rabindra Nath Das,[a] Jan Jamroskovic,[b] Rajendra Kumar,[a] Mattias Hedenström,[a] Nasim Sabouri,[b][c] and Erik Chorell*[a]

Abstract: G-quadruplex (G4) DNA structures are linked to fundamental biological processes and human diseases, which has triggered the development of compounds that affect these DNA structures. However, more knowledge is needed about how small molecules interact with G4 DNA structures. This study describes the development of a new class of bis-indoles (3,3-diindolyl-methyl derivatives) and detailed studies of how they interact with G4 DNA using orthogonal assays, biophysical techniques, and computational studies. This revealed compounds that strongly bind and stabilize G4 DNA structures, and detailed binding interactions which for example, show that charge variance can play a key role in G4 DNA binding. Furthermore, the structure–activity relationships generated opened the possibilities to replace or introduce new substituents on the core structure, which is of key importance to optimize compound properties or introduce probes to further expand the possibilities of these compounds as tailored research tools to study G4 biology.

Introduction

Single-stranded DNA sequences containing four repeats of guanines (normally two-three), bridged by random nucleotides, can fold into G4 DNA structures. In the G4 DNA structure, the guanines bind to each other by Hoogsteen hydrogen bonds and form a quartet of four guanines, one from each guanine stretch, called G-tetrads. The G-tetrads stack on top of each other to form strong π–π interactions and are further stabilized by central monovalent cations, such as potassium or sodium.[1,2] G4 DNA structures can be more stable than the classical double-stranded helical DNA.[3] However, the number of G-tetrads and the length and base composition of the nucleotides that bridge the guanine stretches, called loops, are highly important for the stability of the G4 DNA structure.[4–9]

G4 DNA structures can form when DNA is single-stranded, for instance during cellular processes, such as, DNA replication, transcription, and DNA repair. Furthermore, superhelical stress,[10] molecular crowding,[11] and G4 DNA binding proteins can also favor their formation.[12]

There are about 700,000 predicted G4 DNA structures in the human genome and many of these are evolutionary conserved,[13,14] suggesting that they have important functions. One of the first reported biological implications of G4 DNA structures was their presence at the telomeres, the chromosomal ends.[15] Since then, it has also been shown that G4 DNA structures can inhibit or enhance gene expression and G4 DNA motifs are present in every second human promoter, and most frequently at promoters of oncogenes and regulatory genes.[16,17] Additionally, G4 DNA motifs are also present in the promoters of other organisms.[18–20] G4 DNA structures have thus been linked to fundamental cellular events such as maintenance of the telomeres and transcription, but they also affect DNA replication progression and many proteins and enzymes are reported to bind and/or resolve G4 DNA structures.[15,21–23]

G4 DNA structures are also connected to human diseases such as neurodegenerative diseases and different types of cancers.[24–27] One of the most extensively studied G4 DNA structures in this regard is located in the c-MYC promoter, which is a transcriptional factor affecting cell proliferation that is overexpressed in many human cancers.[28] Mutations that prevent formation of the G4 DNA structure in the c-MYC promoter downregulates transcription of this gene,[28] and selective stabilization of this G4 DNA structure is therefore a potential che-
motherapeutic strategy. The G4-forming region of the c-MYC promoter is a 27 nucleotide sequence, Pu27, that is important for regulating transcription of the c-MYC gene. The Pu27 sequence form parallel polymorphic G4 DNA structures but the predominant G4 DNA structure involved in c-MYC transcriptional regulation is captured in the modified and truncated versions of the Pu27 sequence, Pu24T (G13T) and Pu22 (G14T/G23T) c-MYC G4 DNA structures.

The prevalence and biological relevance of G4 DNA structures as well as their links to human diseases has resulted in a significant interest in the development of compounds that stabilize G4 DNA structures. However, although there are many examples of G4 DNA stabilizing compounds, the reports that fully explore the structure–activity relationships and correlates this to the compound's interactions with G4 DNA structures are scarce. This type of information is of key importance to develop this field of research both towards tailored research tools and to efficiently explore G4 DNA structures as therapeutic targets.

Indoles are frequently occurring in drugs and biologically active compounds and are therefore considered to be privileged structures. Bis-indoles have been shown to efficiently bind and stabilize G4 structures with effects similar or even improved over the most well-known and efficient G4 stabilizing compound Phen-DC3. However, these reported bis-indoles are either linked at the indole nitrogen or in position 2, leaving bis-indoles linked at position 3 unexplored. Furthermore, these studies also show that flexibility is important in the design of G4 ligands and that diindolyl-methyl derivatives can be efficient G4 binders even though their most energetically favorable conformation is non-planar. In this work, we have thus designed and synthesized 3,3-diindolyl-methyl analogues with different side chains to achieve structure–activity relationships (SARs). Additionally, we have developed synthetic methods that allowed us to vary the side chains on the 3,3-bisindolyl analogues in all open positions for all side chains and for a combination of side chains to enhance the SAR study (Figure 1).

To analyze the compounds' abilities to bind and stabilize G4 DNA structures, we used a wide range of methods including fluorescence resonance energy transfer (FRET) melting assay, circular dichroism (CD) spectroscopy, microscale thermophoresis (MST), fluorescence intercalator displacement (FID) assay, nuclear magnetic resonance (NMR), and computational techniques such as molecular dynamics (MD) simulations to understand the molecular details of the results. Overall, this revealed compounds that strongly bind and stabilize G4 DNA structures and key components in the design of these types of compounds and their interactions with G4 DNA structures.

Results and Discussion

Synthesis

To determine how the position and composition of different side chains of the 3,3-diindolyl-methyl affect G4 binding and stabilization, we synthesized different analogues. The nitro-indoles (1a–d; Scheme 1) were the key to investigate the effect of the position and composition of the side chains of the 3,3-diindolyl-methyl derivatives. The 4- and 5-nitro-indoles are commercially available whereas the 6- and 7-nitro-indoles were synthesized according to published procedures in two and five steps, respectively (supporting information). The 7-nitro-indole synthesis also resulted in a 5-bromo substituent for this set of derivatives (which depending on the data generated from these derivatives potentially can be used for late stage functionalization or be dehalogenated). From these four nitro-indoles, attempts to synthesize the di-nitro-3,3-diindolyl-methyl intermediates were performed by reacting the corresponding nitro-indoles with formaldehyde in water and 3 equivalents of acetic acid under refluxing conditions for 12 h. This gave the desired di-nitro-3,3-diindolyl-methyl intermediates (2a–c) in 65–75% yields. Unfortunately, the 5-Bromo-7-nitro indole (1d) did not react under these conditions. However, when the nitro indoles were reacted with formaldehyde using 10% sodium dodecyl sulfate (SDS) in water under microwave irradiation at 100°C for 2 h, all the desired di-nitro-3,3-diindolyl-methyl intermediates (2a–d) were obtained in 88–92% yields. Hydrogenation of the di-nitro-3,3-diindolyl-methyl analogues under Pd/C conditions in methanol at room temperature afforded di-amino-3,3-diindolyl-methyl analogues (3a–d) in 86–96% yields. Next, the four di-amino-3,3-diindolyl-methyl analogues were each coupled with 4 different carboxylic acid substituted side chains using N-[(Dimethylamino)-1H-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide (HATU) as coupling reagent and disopropylethylamine (DIPEA) as base to provide the 16 desired analogues (5aa–ad, 5ba–bd, 5ca–cd, and 5da–dd) in high yields. The choice of side chains was based on both aromatic and aliphatic systems with possibilities to form stacking interactions and/or positive charges under physiological pH or through permanent methylations. Hence, the analogues having quinolines as side chains were also quaternized using methyliodide (MeI) in dimethylformamide (DMF) at 40°C for 24 h to give the four methylated analogues (7aa–da) in 88–96% yields. Unfortu-
nately, methylations of the side chain containing benzo[d]thiazoles (5ab, 5bb, 5cb, and 5db) were not successful even though various methods were applied (summarized in Scheme S1). To further expand the scope of the study, an analogue with the reversed amide (5ea and 7ea) as well as an example with an ethyl substituent on the central carbon (5fa and 7fa) was also synthesized (Scheme 1). In total 24 derivatives (5aa–5fa and 7aa–7fa) with different side chain composition and position on the 3,3-diindolyl-methyl central fragment was prepared.

FRET Melting assay

We next analyzed how the variations of the side chains, both composition and position, affect the compounds ability to stabilize G4 DNA structures by the FRET melting assay. This assay measures the ligand-induced change in melting temperature (ΔTm) of the labeled parallel Pu24T c-MYC G4 DNA structure (see Experimental Section for details, the oligonucleotides used in this study are summarized in Table S1). This showed that both the position and the composition of the side chains on the 3,3-diindolyl-methyl is crucial for an efficient G4 stabilization (Figure 2A and Figure S1). The derivatives with methylated quinoline side chains in position 5 and 6 (7ba and 7ca) clearly gave the most efficient stabilization with a dramatic difference compared to analogues with substituents in position 4 and 7 (7aa and 7da). Furthermore, the methylated quinoline side chain in the 5- and 6-substituted derivatives (7ba and 7ca) proved to be of utmost importance for an efficient G4 stabilization when compared to their homologues with non-methylated quinoline side chains, suggesting that the charge is important for the quinoline side chains. This was supported by the derivatives with neutral benzo[d]thiazoles side chains (5ab–5db) that showed weak stabilization. However, the charge alone could not be responsible for the observed effects as the pipеразине and morpholine derivatives (5ac–5dc and 5ad–5dd, respectively), which are positively charged in the physiological pH used in the experiments, only showed weak stabilization. The pipеразине and morpholine side chains lack the ability to form π-stacking interactions which suggest that this is important and cannot in full be compensated for by the potential electrostatic interactions of these derivatives. Reversing the amides (7ea compared to 7da) did not have any major impact on the compounds ability to stabilize G4 DNA in this assay. Importantly, all compounds showed good selectivity for G4 DNA structures over double-stranded DNA (Figure S2). A potential problem with the FRET assay is the risk that compounds can interfere with the FRET process and thereby give false positive/negative results. However, these effects should appear immediately and not be temperature-dependent. None of the tested compounds displayed a temperature-independent effect on the FRET process thus suggesting that they do not affect the fluorophores or disrupt the G4 DNA structure (except 7fa with a central ethyl group which affected the FRET process likely by interacting with the carboxytetramethylrhodamine (TAMRA) fluorophore, which can be the reason for the decreased stabilization ability observed for this compound compared to 7ca).
amount of full-length products. Importantly, compounds that bind DNA non-selectively and therefore cause DNA replication arrest, can also be detected when running the experiments in parallel with an on-G4 control template. We first performed a primary screen that showed a clear G4 stabilization by 7ba–7fa as indicated by a strong replication pausing one or two nucleotides before the first G-tract in the G4 structure (Figure 2B, denoted with asterisks; Figure S3B). 7ba–7fa also displayed a clear selectivity for G4 DNA compared to the non-G4 control template (Figure 2 versus S4) although they also slightly suppressed primer utilization for both the G4 and non-G4 DNA (Figure 2B, Figures S3A, S4A, and S4C). In line with the FRET melting studies, none of the tested neutral 3,3-diindolylmethyl derivatives (5) showed G4 stabilization in this assay (Figure 2). Both the FRET melting studies and this assay clearly showed that 7ba induced the strongest stabilization (Figure 2). The 4- and 7-substituted derivatives 7aa and 7da both have low effects, which likely can be attributed to steric hindrance to adopt a conformation that match the G-tetrad and also suggest that the 5-bromo substituent likely is not of key importance for the effect of 7da. In the Taq polymerase stop assay, we also detected strong pausing and reduced amount of full-length products by 7fa, however this was not found with the FRET melting assay (Figure 2). Furthermore, both the central ethyl substituent and reversing the amide had only mild impact on the stabilization ability (7fa versus 7ca and 7ea versus 7da, respectively) in this assay.

Next, we performed dose-response Taq polymerase stop assays to determine the efficiency and selectivity of 7aa, 7ba, and 7ca by calculating the IC50 values (Table 1, Figures S5 and S6). All three compounds showed a clear dose response, and the IC50 for the G4 DNA template was 1.5, 0.17, and 0.24 μM for 7aa, 7ba, and 7ca, respectively (Table 1, Figure S5). For the non-G4 DNA template, the IC50 values were >5, 2, and 1.7 μM, respectively (Table 1, Figure S6). These data demonstrate the lowest IC50 values for 7ba and 7ca, and a strong selectivity by both compounds for the G4 DNA template compared to the non-G4 DNA.

| Ligands | ΔTm (°C) | ΔTm (°C) | IC50 (μM) | Kd (μM) | Apparent Kd (μM) |
|---------|----------|----------|-----------|--------|-----------------|
| 7aa     | 0.7 ± 0.2| 1.8 ± 0.3| 5.3       | 1.55 ± 0.13| >5              |
| 7ba     | 4.2 ± 0.8| 17.5 ± 1.5| 4.0       | 0.17 ± 0.06| 2.01 ± 0.3      |
| 7ca     | 0.8 ± 0.1| 10.4 ± 1.0| 3         | 0.24 ± 0.06| 1.72 ± 0.07     |
| 5bac    | 0        | 6.0 ± 0.3| n.d.      | n.d.     | n.d.            |
| 7bac    | 1.5 ± 0.8| 12.4 ± 0.9| >12       | 0.19 ± 0.015| 4.02 ± 0.45   |

[a] Using the FRET melting assay at the tabulated compound concentrations (see also Figure S1 and S10). [b] Using CD melting (see also Figure S8). [c] Using the Taq polymerase stop assay (see also Figure S5 and S6). [d] Using MST (see also Figure S11). [e] Using FID assay (see also Figures S12 and S13). n.d.-not determined.

Figure 2. (A) Evaluation of the compounds ability to stabilize the Pu24T c-MYC G4 DNA structure using the FRET assay (at 2 μM ligand concentration). Tm in absence of ligands of Pu24T is 62.7 ± 0.3 °C. (B) Primary screening of the compounds (at 1 μM ligand concentration) by Taq-polymerase stop assay using Pu24T c-MYC G4 DNA template. *indicates pausing sites. Grey arrow represents full-length product, black arrow represent primer—start of the reaction. (C) Relative quantification of full-length product in each reaction. Error bars correspond to SD of at least three independent experiments.
Synthesis of asymmetrical compounds

Based on the data above, the positioning of the side chains on the 3,3-diindolyl-methyl central fragment proved to be highly important with position 5 giving the most efficient G4 DNA stabilizing compounds in both assays. Derivative 7ba with methyalted quinoline side chains in position 5 was the most promising derivative and other side chains in position 5 did not result in compounds with any G4 stabilizing capacity at the tested concentrations. However, we hypothesized that not both side chains are required for an efficient binding and stabilization, which thus would open for the possibility to use one of the side chain positions for example, improving compound properties or reaching additional electrostatic interactions with the phosphate backbone. Hence, a combined derivative with one quinoline and one piperazine side chain in position 5 of the 3,3-diindolyl-methyl central fragment was synthesized (Scheme 2).

Many different strategies were tested to achieve these derivatives (Scheme S2). However, the successful synthetic method (Scheme 2) started with a coupling reaction between one amino group of 3,3'-methylenebis(1H-indol-5-amine) 3b with 4-(4-methylpiperazin-1-yl)butanoic acid (4a), quinoline-3-carboxylic acid (4c) using HATU and DIPEA conditions to afford the unsymmetrical 5bac in 70% yield. Subsequent methylation gave the tri methylated compound 7bac in 88% yield.

G4 stabilization by the asymmetrical analogues

Next, the 3,3-diindolyl-methyl derivatives with one quinoline and one piperazine side chain (5bac and 7bac) were tested for their ability to affect the progression of the Taq DNA polymerase with Pu24T c-MYC G4 DNA and non-G4 DNA (Figure S7). Similar to the other 5 and 7 analogues, the charged 7bac stabilized the G4 structure whereas the neutral 5bac did not (Figure S7). To test the efficiency of 7bac to selectively stabilize G4 structures, we also performed the dose-response Taq polymerase stop assay (Figures S5 and S6). 7bac displayed a clear dose response and efficiently blocked the Taq polymerase 1–2 nucleotides before the G4 DNA structure. In fact, the 3,3-diindolyl-methyl derivative with one quinoline and one piperazine side chain, 7bac, displayed an IC50 of 0.2 μM, showing that it is equally active as the derivative with two methylated quinolines (7ba) (Table 1). Furthermore, this asymmetric analogue demonstrates an impressive selectivity for the G4 template over the non-G4 template (20-fold).

To further confirm that these derivatives stabilize the Pu24T c-MYC G4 DNA structure, we used thermal CD spectroscopy. Thermal CD melting experiments again showed that the compounds with methylated side chains are able to stabilize the G4 DNA structure from thermal melting, whereas the derivative with only piperazine side chains (5ac) did not have any effect (Figure S8). Importantly, the CD studies also showed that the compounds did not affect the structural folding of the Pu24T G4 DNA structure (Figure S9). However, this assay did not give the same internal order of efficiency as the Taq DNA polymerase stop assay and showed that 7bac (with one methylated quinoline and one piperazine side chain) stabilized the G4 DNA structure much more efficient than 7ba (with two methylated quinolines). This can likely be explained by the difference in the compound’s interactions with the G4 DNA (stacking versus electrostatic interactions) which may affect these assays differently. Furthermore, the FRET melting assay showed similar results as the Taq polymerase stop assay with high melting temperatures for both 7ba and 7bac (Table 1, Figure S10). The FRET assay further showed intermediate stabilization for 7ca whereas both 5bac (the non-methylated version of 7bac) and 7aa were unable to stabilize the Pu24T c-MYC G4 DNA structure from thermal denaturation.

Binding affinity studies

To investigate the compounds (7aa, 7ba, 5bac, and 7bac) binding affinity to the Pu24T c-MYC G4 DNA structure, we used MST (Table 1, Figure S11). The 3,3-diindolyl-methyl derivative 7ba with two methylated quinoline side chains in position 5 was the strongest binder in this assay with a Kd of 0.49 μM, whereas 7aa with two methylated quinoline side chains in position 4 did not reach saturation at the highest concentration (10 μM). This again emphasize the importance of the correct positioning of the side chains on the central fragment for an efficient binding to the G4 DNA structure. As expected, the methylated 7bac (with one quinoline and one piperazine side chain) displayed a much stronger binding affinity (3.05 μM) compared to the non-methylated homolog 5bac which did not bind to the Pu24T c-MYC G4 DNA in this assay. However, the difference in binding affinity between 7ba and 7bac was surprisingly high. Therefore, the FID assay with thiazole orange (TO) was used to confirm these binding affinities (Table 1, Figures S12 and 13). This assay ranked the compounds in the same internal order, but showed a more similar binding affinity (1.4 μM and 1.7 μM for 7ba and 7bac, respectively).

Scheme 2. Synthesis of asymmetrical 3,3-diindolyl-methyl analogues. Reaction conditions: a) 4-(4-methylpiperazin-1-yl)butanoic acid (4a), quinoline-3-carboxylic acid (4c), HATU, DIPEA, DMF, rt, 12 h, 70% yield; b) Mel (6a), DMF, 40 °C, 24 h, 88% yield.
NMR studies

To further probe the compounds’ abilities to bind the Pu24T c-MYC G4 DNA structure, we performed NMR titration studies with 7aa, 7ba, 5bac, and 7bac, ranging from 0.1–5 equivalents and using the peaks in the imino-region of the spectra as probes for G4 interactions (Figure 3). This showed some clear differences between the compounds; 7bac induced the biggest changes on the G4 structure with a strong effect even at a 0.1:1 compound to DNA ratio, and an almost complete disappearance of NMR signals at the higher concentrations was found. Based on the other assays with 7bac, it is unlikely that this compound induced unfolding of the G4 DNA. Instead, the most likely explanation for the observed effect is exchange between free and bound form of the G4 DNA on an intermediate time-scale, resulting in line-broadening. Compound 7aa also induced strong effects on the G4 DNA structure at substoichiometric concentrations whereas 7ba required almost equimolar concentrations of compound to DNA before a clear effect could be observed. However, at these concentrations of 7ba, a new set of peaks starts to appear that was not observed with the other compounds, suggesting that 7ba have a stronger binding, resulting in a shift from intermediate to slow exchange on the NMR time-scale where peaks from both free and bound form of the DNA are observed. As expected, 5bac did not induce any big changes. However, at equimolar and higher amounts of 5bac compared to DNA, a clear effect on the guanines at the 5'-G-tetrad was clearly visible indicating that this compound indeed binds G4 DNA structures although it does not stabilize them to the same extent as the other compounds. All compounds primarily affected the guanines on the 5'-G-tetrad although almost all guanines were affected at the higher concentrations of 7aa, 7ba, and 7bac.

Molecular dynamics simulations

We next used molecular dynamics (MD) simulations to understand the molecular details of the observed G4 DNA binding and stabilization. This will also give valuable information about the binding mode of the 3,3-diindolyl-methyl derivatives with G4 DNA. Based on the NMR results, we modeled compounds 7aa, 7ba, 5bac, and 7bac at the 5'-G-tetrad of the Pu24T c-MYC G4 DNA structure and performed 1 μs MD simulations of these complexes. The sampled binding modes for these compounds were subsequently clustered using principal component analysis (PCA).

Five clusters were obtained for 7aa and contrary to the expected binding mode, all these clusters showed a partially self-stacked conformation of 7aa with one indole stacking on a quinoline (Figure 4, Figure S14, and Table S2). This conformation reduced the stacking interaction of 7aa with the top G-tetrad due to the loss of the planar compound conformation. The self-stacked conformation of 7aa was also present in its unbound form thus highlighting the rigidity of this conforma-
did not give the same stable binding that is not in-
and T was also sandwiched www.chemeurj.org and was also able to rotate on the top of the G-
tetrad.

The Authors. Published by Wiley-VCH Verlag GmbH &Co. KGaA, Weinheim

Figure 4. Representative binding poses of compounds during MD simula-
tions. The 5'-G-tetrad (light blue), nucleotides flanking the G4 DNA structure (orange), and compounds (ball-stick model) from the largest cluster central structure 7aa, 7ba, 5bac, and 7bac. The interactions of 7bac with the DNA backbone is highlighted with a space filling model. The potassium ion (cen-
tral purple sphere) is also shown. The binding energies are tabulated in Table S6.

Overall, the obtained binding energy suggests that 7bac is the strongest binder while 5bac is a very weak binder (Table S6). However, the computed binding energy does not in-
clude entropic contribution and therefore, these cannot be di-
rectly compared with experimental values.

Additionally, when the quinoline side chain was methylated, the partial charge variance significantly decreased (Figure S18). This reduction in partial charge variance strengthen the stack-
ing interaction with the guanines in the G-tetrad because gua-
nine also possess large charge variance and two groups with large charge variance will thus repulse each other which will destabilize the stacking interaction. Therefore, a low partial charge variance is preferable for a strong stacking interaction with the guanines in the G-tetrad, as observed for the methy-
lated quinoline in 7bac compared to the non-methylated quin-
one in 5bac.

Selectivity studies

Finally, we investigated if 7ba and 7bac were able to bind and stabilize other types of G4 DNA structures in addition to the Pu24T c-MYC G4 DNA structure. A selection of 11 different G4 DNA structures were screened using the FRET assay and the re-

results showed that 7ba and 7bac efficiently bind to many types of G4 structures (Figure S15). The c-MYC Pu22 and Pu24T G4 DNA structures are very similar and it is thus not surprising that both 7ba and 7bac effectively stabi-

lize both these structures. Furthermore, the compounds were also able to stabilize several other G4 DNA structures correlat-
ed to oncogenes, such as the c-Kit and Bcl-2 G4 DNA struc-
tures.[38,39] This can be a valuable attribute as it has been pro-
posed that it in fact can be more effective to target several G4 DNA structures simultaneously in cancer therapy.[40]
To further study the selectivity of 7ba and 7bac to G4 DNA, we investigated if double-stranded DNA can compete out the binding of 7ba and 7bac to the G4 DNA structure. We therefore performed the FRET melting assay with G4 DNA, compound, and increasing concentrations of double-stranded DNA. This again showed the compound’s selectivity for G4 DNA, as even 50-fold excess of double-stranded DNA hardly affected $\Delta T_m$ in the FRET melting studies (Figure 5B).

**Conclusions**

Bis-indoles are known to have the potential to efficiently bind and stabilize G4 DNA. In this work, we have developed a new bis-indole core structure and evaluated the effect of both the position and chemical composition of the side chains attached to this scaffold. Multistep synthetic method developments generated convenient methods to synthesize the target compounds, starting from readily available starting materials. Using these methods, a set of 26 target compounds were synthesized with varying substituents both in terms of their position on the core structure and their chemical composition. The compounds were evaluated using an array of different biochemical and biophysical techniques (FRET melting assay, CD, NMR, Taq polymerase stop assay, MST, FID) as well as computational techniques (MD simulations and charge variance calculations). This revealed compounds that bind and stabilize G4 DNA structures with well-defined dose-response curves and similar or even improved selectivity and activity compared to previously reported bis-indoles. Furthermore, this work also resulted in important structure–activity relationships that were correlated to the compound’s interactions with the G4 DNA to understand why certain structural motifs are so important for the interactions with G4 DNA structures. For example, the methylated quinoline side chains generated the most efficient bis-indoles in terms of G4 binding and stabilization, and the methylation of the quinoline proved crucial for this activity. The MD simulations and charge variance calculations show that the reason for this does not lie in the possibility for electrostatic interactions but rather the formation of a substituent with low charge variance that can stack efficiently on the top 5' G-tetrad. However, even though this charged quinoline is required for binding, it did not prove to be crucial to have two of these substituents as one could be replaced, while still retaining most of the binding and stabilization abilities. Moreover, the position of the substituents proved crucial for both the selectivity and stabilization ability with substitutions in position 5 being preferred as this has the ability to adopt a conformation that place the substituents in the right position to bind the G-tetrad. This type of detailed structure–activity relationships connected to a deep understanding of the compounds G4 DNA interactions open the possibilities to replace or introduce new substituents. This is of key importance to improve pharmacokinetic properties or to broaden the use of these G4 binding compounds through for example, the introduction of chemical handles for pull-down experiments or fluorophores and, ultimately, to better understand G4 biology and its therapeutic potential.

**Experimental Section**

**Selected synthesis**

Procedure for the preparation of $N,N^\prime$-(3,3'-methylene-bis[1H-indole-4,3-diyl])bis(quinoline-3-carboxamide) (ECH-108) (5aa): HATU (322 mg, 0.85 mmol) was added to the solution of quinoline-3-carboxylic acid (4a) (140 mg, 0.80 mmol) in a dry DMF (3 mL) and the reaction mixture was stirred at room temperature until the solids were dissolved completely. DIPEA (155 mg, 1.2 mmol) was thereafter slowly added to the reaction mixture that was stirred for

---

Find the full text in the online version at [doi.org/10.1002/chem.202000579](https://doi.org/10.1002/chem.202000579)
10 min followed by slow addition of di-amine 3a (110 mg, 0.40 mmol) in DMF (3 mL). The mixture was stirred at room temperature under nitrogen atmosphere for 12 h. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured into ice and filtered through sintered funnel. The solid was dried under vacuum. The pure compound 5a was isolated in 92% yield as a light-yellow solid. 1H NMR (600 MHz, [D$_6$]DMSO): $\delta$ = 10.93 (d, J = 2.5 Hz, 2H), 10.06 (s, 2H), 9.31 (s, 2H), 8.44 (d, J = 2.3 Hz, 2H), 8.04 (d, J = 8.4 Hz, 2H), 7.85 (t, J = 8.4 Hz, 2H), 7.69 (d, J = 8.1 Hz, 2H), 7.61 (t, J = 7.3 Hz, 2H), 7.25 (d, J = 7.7 Hz, 2H), 7.18 (d, J = 8.1 Hz, 2H), 7.03 (t, J = 7.8 Hz, 2H), 6.89 (d, J = 2.3 Hz, 2H), 4.62 ppm (s, 2H); 13C NMR (150 MHz, [D$_6$]DMSO): $\delta$ = 164.84, 149.22, 138.60, 136.11, 131.85, 130.28, 129.95, 128.47, 127.67, 127.57, 126.80, 124.54, 124.21, 121.47, 121.21, 116.37, 114.09, 110.23, 23.70 ppm; MS (ES mass): m/z 587.3 (M+1); HRMS: m/z calcd for C$_{38}$H$_{38}$N$_{10}$O$_{27}$+ 587.2190, found 587.2180; Purity: 96.8% (according to HPLC).

Procedure for the preparation of N,N’-(3,3’-methylenebis(1H-indole-4,3-diyl))bis(phenol)diazotized-thiosemicarbazide (ECD-109) (5ab): HATU (322 mg, 0.85 mmol) was added to the solution of benzodithiazole-2-carboxylic acid (4b) (143 mg, 0.80 mmol) in a dry DMF (3 mL) and the reaction mixture was stirred at room temperature until the solids were dissolved completely. DIPEA (155 mg, 1.2 mmol) was thereafter slowly added to the reaction mixture that was stirred for 10 min followed by slow addition of di-amine 3a (110 mg, 0.40 mmol) in DMF (3 mL). The mixture was stirred at room temperature under nitrogen atmosphere for 12 h. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured into ice and filtered through sintered funnel. The solid was dried under vacuum. The pure compound 5ab was isolated in 89% yield as a light-yellow solid. 1H NMR (600 MHz, [D$_6$]DMSO): $\delta$ = 11.02 (s, 2H), 10.23 (s, 2H), 8.12 (d, J = 7.7 Hz, 2H), 7.55 (dd, J = 8.2, 4.5 Hz, 4H), 7.52–7.47 (m, 4H), 7.28 (d, J = 8.1 Hz, 2H), 7.12 (t, J = 7.9 Hz, 2H), 6.92 (d, J = 2.3 Hz, 2H), 4.74 ppm (s, 2H); 13C NMR (150 MHz, [D$_6$]DMSO): $\delta$ = 164.52, 158.14, 152.70, 138.72, 136.66, 129.74, 127.33, 127.29, 124.67, 123.21, 121.61, 120.61, 113.61, 113.50, 110.11, 24.52 ppm; MS (ES mass): m/z 599.2 (M+1); HRMS: m/z calcd for C$_{38}$H$_{38}$N$_{10}$O$_{27}$+ 599.1318, found 599.1296; Purity: 99.1% (according to HPLC).

Procedure for the preparation of N,N’-(3,3’-methylenebis(1H-indole-4,3-diyl))bis(4-(4-methylpiperazin-1-yl)butanamide) (ECD-110) (5ac): HATU (322 mg, 0.85 mmol) was added to the solution of 4-(4-methylpiperazin-1-yl)butanamide (4c) (149 mg, 0.80 mmol) in a dry DMF (3 mL) and the reaction mixture was stirred at room temperature until the solids were dissolved completely. DIPEA (155 mg, 1.2 mmol) was thereafter slowly added to the reaction mixture that was stirred for 10 min followed by slow addition of di-amine 3a (110 mg, 0.40 mmol) in DMF (3 mL). The mixture was stirred at room temperature under nitrogen atmosphere for 12 h. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured into ice and filtered through sintered funnel. The solid was dried under vacuum. The pure compound 5ac was isolated in 95% yield as a light-yellow brown solid. 1H NMR (600 MHz, [D$_6$]DMSO): $\delta$ = 10.97 (s, 2H), 10.37 (s, 2H), 9.72 (s, 2H), 9.26 (s, 2H), 8.53 (d, J = 8.9 Hz, 2H), 8.37 (m, 2H), 8.29 (d, J = 8.9 Hz, 2H), 8.08 (t, J = 7.6 Hz, 2H), 7.13 (d, J = 8.0 mmol, 2H), 6.85–6.82 (m, 4H), 6.78 (d, J = 7.4 Hz, 2H), 4.67 (s, 6H), 4.40 ppm (s, 2H); 13C NMR (150 MHz, [D$_6$]DMSO): $\delta$ = 161.37, 149.43, 144.46, 138.65, 138.02, 136.86, 136.11, 136.34, 128.39, 128.72, 127.08, 124.67, 123.53, 110.11, 45.73, 34.39 ppm; MS (ES mass): m/z 615.2 (M–1); HRMS: m/z calcd for C$_{38}$H$_{38}$N$_{10}$O$_{27}$+ 615.2851, found 615.2617; Purity: 98.7% (according to HPLC).

Procedure for the preparation of 3,3’-((3,3’-methylenebis(1H-indole-4,3-diyl))bis(azanedi-yl))bis(carbonyl)bis(1-methylquinolin-1-ium) iodide (ECD-112) (7aa): To the compound 5a (30 mg, 0.05 mmol) dissolved in DMF (2 mL) was added methylidode (6a) (212 mg, 1.5 mmol) drop wise at room temperature under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 24 h. The progress of the reaction was monitored by LC-MS. Upon completion, the reaction mixture was precipitated by slow addition of acetone. The precipitate was filtered through a sintered funnel and washed with diethyl ether and acetone. The solid was dried under vacuum to obtain pure compound 7aa as a light brown solid in 93% yield. 1H NMR (600 MHz, [D$_6$]DMSO): $\delta$ = 10.97 (s, 2H), 10.37 (s, 2H), 9.26 (s, 2H), 9.72 (s, 2H), 8.53 (d, J = 8.9 Hz, 2H), 8.37 (m, 2H), 8.29 (d, J = 8.9 Hz, 2H), 8.08 (t, J = 7.6 Hz, 2H), 7.13 (d, J = 8.0 mmol, 2H), 6.85–6.82 (m, 4H), 6.78 (d, J = 7.4 Hz, 2H), 4.67 (s, 6H), 4.40 ppm (s, 2H); 13C NMR (150 MHz, [D$_6$]DMSO): $\delta$ = 161.37, 149.43, 144.46, 138.65, 138.02, 136.86, 136.11, 136.34, 128.39, 128.72, 127.08, 124.67, 123.53, 110.11, 45.73, 34.39 ppm; MS (ES mass): m/z 615.2 (M–1); HRMS: m/z calcd for C$_{38}$H$_{38}$N$_{10}$O$_{27}$+ 615.2851, found 615.2617; Purity: 98.7% (according to HPLC).
Methods

Folding of G4 structures for FRET study: Synthetic oligonucleotides for FRET study were purchased from Eurofins Genomics. Stock solutions were prepared in water at 100 μM concentration. The sequences used are listed in supplementary Table S1. All the oligonucleotides except Pu22 were prefolded in 10 mM lithium cacodylate buffer (pH 7.4), with 10 mM KCl and 90 mM LiCl by heating for 5 min at 95 °C and then quick cooling on ice. Pu22 was folded in 10 mM lithium cacodylate buffer (pH 7.4), with 2 mM KCl and 98 mM LiCl.

FRET melting assay: The fluorescence resonance energy transfer (FRET) occurs between two dyes (5’-FAM as donor and 3’-TAMRA as acceptor) linked at both extremities of a DNA oligonucleotide. When the oligonucleotides are folded into G4 structures, the donor and acceptor are in close proximity, which results in an energy transfer from the donor to the acceptor. This process can be detected by a reduction in the fluorescence emission of the donor. Fluorescence emission of the donor is recovered when the temperature increment triggers the thermal denaturation of the G4 structure. The experiments were performed in a Bio-rad CFX96 real-time PCR device at temperatures from 10 to 95 °C with a heating rate of 1.5 °C·min⁻¹ using a 492-nm excitation wavelength and a 516-nm detection wavelength in 96-well plates. Each condition was tested in duplicate and analysis of the data was carried out using Excel and Origin 8 software. In each well, 200 nM of labelled oligonucleotide was heated in the presence or absence of the ligand (and with or without the competitor dsDNA) at the specified concentrations. Emission of 5’-FAM was normalized between 0 and 1, and the melting temperature (Tm) is defined as the temperature at which 50% of the G4 structures are denatured (the temperature when the normalized emission was 0.5). The stabilization (ΔTm) was calculated from comparison of Tm of the fluorescently labeled oligonucleotide in the presence or absence of the ligand.

Taq-polymerase stop assay: 1 μM TET-labeled primer was annealed to 1.25 μM template DNA in 50 mM KCl by heating the reaction at 95 °C for 5 min and slowly cool down overnight to room temperature. Folding of the G4 structure and primer annealing proceeded simultaneously. For each reaction in the screening procedure, 40 nM annealed DNA was incubated with 1 μM compounds or 1 μM DMSO (control reaction) for at least 30 min in 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 1.5 mM MgCl₂, and 200 μM dNTPs at room temperature. DNA synthesis was started by the addition of 0.625 U of recombinant Taq DNA-polymerase (Thermo Scientific) into the reaction mixture (10 μL), and incubated 30 min at 50 °C. The reaction was stopped by addition of one volume of stop solution (95% formamide, 20 mM ethylenediaminetetraacetic acid (EDTA), and 0.1% bromophenol blue) into the reaction. A total of 8 μL of the mixture was loaded into a 10% polyacrylamide gel containing 8% urea, 25% formamide, and 1x tris/borate/ethylenediaminetetraacetic acid (TBE). The gel was visualized with a Typhoon Scanner 9400 (GE Healthcare) at λ = 532 nm and quantified with the Image Quant TL 8.1 software (GE Healthcare). Quantifications are displayed as an average of two experiments ± absolute error. For the determination of IC₅₀ values, the DNA was incubated with different concentrations (5, 2, 0.8, 0.32, 0.13, 0.05, 0.025, and 0.008 μM) of the tested compounds or 5% (v/v) DMSO. IC₅₀ values were calculated using GraphPad Prism software.

Chem. Eur. J. 2020, 26, 9561 – 9572 www.chemeurj.org
9570 © 2020 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
were calculated by fitting the full-length products into the dose response function in the OriginPro 2016 software. All reactions were independently repeated three times and standard deviation was calculated.

**CD melting experiment:** 50 μM folded oligonucleotides diluted to 5 μM concentration into 10 mM Tris (pH 7.5); 10 mM KCl and 10 μM compounds or 1.25% DMSO were used for CD melting studies. A quartz cuvette with a path length of 1 mm was used for the measurements by JASCO-720 spectropolarimeter (Jasco International Co. Ltd.). First, CD spectra were recorded at 25 °C over λ = 230–350 nm with an interval of 0.2 nm and a scan rate of 100 nm/min⁻¹. The corresponding buffer was used alone for baseline correction of each measurement. Melting curves were recorded at 264 nm between 25–95 °C at a speed of 1 °C/min⁻¹. Melting temperature (Tm) is defined as the temperature at which 50% of the G4 structures are unfolded. Tm values were estimated by fitting the melting curves into a dose response function using the OriginPro 2016 software.

**Fluorescence intercalator displacement experiments:** The experiments were performed at 25 °C on a Jasco FP-6500 spectrophotometer equipped with a temperature controller. 0.25 μM of prefolded G4-DNA in 10 mM potassium phosphate (pH 7.4), 100 mM KCl buffer was mixed with 0.50 μM Thiazole Orange (TO) and incubated for 2 minutes before the fluorescence spectrum was recorded. The lifetime (τem) was 0.51 ns; τem = 510–650 nm. Then ligands were added to the mixture stepwise with a 2 min equilibration period, and the fluorescence spectra were recorded. The percentage of TO displacement was calculated from the fluorescence intensity (F) at the emission maxima, using Equation (1):

\[
\text{Percentage of TO displacement} = 100 - \left( \frac{F \times 100}{F_0} \right)
\]

(1)

where, F0 is the initial fluorescence intensity of TO bound to G4-DNA. The percentage of TO displacement was plotted as a function of the concentration of added ligands and DC50 is determined. The association binding constant (Ka) of the ligands were calculated from Equation (2) using \( \kappa_{\text{BIND}}^2 \) as 5.01 × 10⁻¹⁰ M⁻¹:s⁻¹:

\[
\kappa_{\text{BIND}}^2 = \frac{K_{\text{a}} \times [\text{TO}]}{[\text{ligand}]_{50}}
\]

(2)

**Microscale thermophoresis (MST):** The Pu24T G4 DNA sequence with a 5'-CY5 label was folded in KCl buffer (10 mM phosphate, 100 mM KCl, pH 7.4) by heating at 95 °C for 5 min and then cooling to room temperature. All the experiments were performed in 10 mM phosphate pH 7.4, 100 mM KCl, 0.05% Tween20 and 4% BSA. The labelled DNA concentration was kept constant at 25 nM and ligand concentration is varied from 0.15 mM to 10 mM (fourteen 1:1 dilutions). The samples were loaded into standard MST graded glass capillaries and MST experiment is performed using Monolith NT.115 (Nano Temper, Germany) with 40% LED power. Data was analyzed using the Nano Temper analysis software and plotted in OriginPro 8.

**Nuclear magnetic resonance:** The G4 DNA stock solutions was prepared by folding 100 μM c-MYC Pu24T in 10 mM potassium phosphate buffer (pH 7.4) and 35 mM KCl by heating to 95 °C and slowly cooling to room temperature overnight. 10% D2O and 10% [D2]DMSO was added to the DNA stock solutions, yielding a final DNA concentration of 82 μM. NMR samples were prepared by sequential addition of 7aa, 7ba, 5bac, and 7bac from 20 mM DMSO stock solutions to 220 μL of the DNA solution which was then transferred to 3 mm NMR tubes. Control samples with Pu24T c-MYC G4 DNA with and without 10% DMSO was also performed to verify that DMSO did not have a significant effect on the DNA structure. All spectra were recorded at 298 K on a Bruker 850 MHz Avance III HD spectrometer equipped with a 5 mm TCI cryoprobe. Excitation sculpting was used in the 1D 1H experiments, and 256 scans were recorded. Processing was performed in Topspin 3.5 (Bruker Biospin, Germany).

**G4 complex modeling:** For Pu24T c-MYC G4 DNA, structure coordinates were downloaded from PDB with PDB-ID 2M9N.[43] From the Pu24T structure, the bound ligand was removed. In the next step, three-dimensional structural coordinates of 7aa, 7ba, 5bac, and 7bac were generated using Avogadro package.[42] To model the Pu24T c-MYC G4 DNA complexes, docking was performed at the 5˚ terminal G-tetrad using Autodock Vina.[43] Two potassium ions were preserved inside the G4 channel to maintain its stability during the simulations.

**Molecular dynamics simulations:** All G4-compound complexes were prepared for molecular dynamics simulations using GROMACS[44] by placing these at the center of a periodic dodecahedron box and solvating with water molecules. Subsequently, the system was neutralized by adding an excess of 0.100 M KCl using the GROMACS tools. For the DNA, Amber95SB[45] with PARAMS1[46] improvements were used as force-field parameters. For water, the TIP3P model[47] was considered while ion parameters were taken from the following reference[48]. For 7aa, 7ba, 5bac, and 7bac, at first, their geometry was optimized and ESP was calculated with HF/6-31G* basis set using Gaussian-16[49] and subsequently the partial atomic charges were calculated with the RESP method using the AmberTools package.[50] All four compounds’ force-field parameters were generated from GAFF using the AmberTools package and converted to GROMACS format using acpype script.[51] Subsequently, MD simulations were performed using GROMACS package.[44] Parameter settings for all these stages were previously described in the following reference[50] and length of the MD simulations were 5*200 ns for each compound. All trajectories were merged for respective complexes, processed, and further used for the analysis.

Conformational clustering for each compound bound to their corresponding G4 DNA structure was performed with the gmx cluster-ByFeatures tool using PCA based conformational clustering (https://github.com/rjdkmr/gmx cluster-ByFeatures). Subsequently, the first 50 frames of each cluster were considered for binding energy calculation using the g mmpbsa tool.[52] The obtained MD trajectories were visualized and images were rendered using VMD.[53]

**Acknowledgements**

Work in the Chorell lab was supported by the Kempe foundations (SMK-1632) and the Swedish Research Council (2017-05235). Work in the Sabourli lab was supported by Knut and Alice Wallenberg Foundation (KAW2015-0189), the Swedish Research Council (VR-MH 2018-2651), the Swedish Cancer Society (CAN2019/126), and the Medical Faculty of Umeå University. The MD simulations were performed on resources provided by the Swedish National Infrastructure for Computing (SNIC) at HPC2N Umeå, Sweden. We also thank the Knut and Alice Wallenberg foundation program NMR for Life (www.nmforlife.se) for NMR spectroscopy support.
Conflict of interest

The authors declare no conflict of interest.

Keywords: bis-indole • DNA structures • drug design • G-Quadruplexes • nitrogen heterocycles

[1] D. Sen, W. Gilbert, Nature 1990, 344, 410–414.
[2] J. R. Williamson, M. K. Raghuraman, T. R. Cech, Cell 1989, 59, 871–880.
[3] A. N. Lane, J. B. Chaires, R. D. Gray, J. O. Trent, Nucleic Acids Res. 2008, 36, 5482–5515.
[4] M. P. Cheng, Y. Cheng, J. Y. Hao, G. Q. Jia, J. Zhou, J. L. Mergny, C. Li, Nucleic Acids Res. 2018, 46, 9264–9275.
[5] A. Risitano, K. R. Fox, Nucleic Acids Res. 2004, 32, 2598–2606.
[6] A. Guédin, J. Gros, P. Alberti, J. L. Mergny, Nucleic Acids Res. 2010, 38, 7858–7868.
[7] R. Tippana, W. K. Xiao, S. Myong, Nucleic Acids Res. 2014, 42, 8106–8114.
[8] P. A. Rachwal, T. Brown, K. R. Fox, FEBS Lett. 2007, 581, 1657–1660.
[9] G. Sattin, A. Arteze, M. Nadai, G. Costa, L. Parrota, S. Alcara, M. Palumbo, S. N. Richter, PLoS One 2013, 8, e84113.
[10] D. Sun, L. H. Hurley, J. Med. Chem. 2009, 52, 2863–2874.
[11] D. Miyoshi, H. Harimata, N. Sugimoto, J. Am. Chem. Soc. 2006, 128, 7957–7963.
[12] E. N. Nikolova, E. Kim, A. A. Wise, P. J. O’Brien, I. Andricioaei, H. M. Al-Hasimi, Nature 2011, 470, 498–484.
[13] V. S. Chambers, G. Marisco, J. M. Boutilier, M. Di Antonio, G. P. Smith, S. Balasubramanian, Nat. Biotechnol. 2015, 33, 877–881.
[14] G. Marisco, V. S. Chambers, A. B. Sahakyan, P. McCauley, J. M. Boutilier, M. Di Antonio, S. Balasubramanian, Nucleic Acids Res. 2019, 47, 3862–3874.
[15] D. Rhodes, H. J. Lips, Nucleic Acids Res. 2015, 43, 8627–8637.
[16] J. Eddy, N. Maizels, Nucleic Acids Res. 2006, 34, 3887–3896.
[17] J. L. Huppert, S. Balasubramanian, Nucleic Acids Res. 2007, 35, 406–413.
[18] J. A. Capra, K. Paeschke, M. Singh, V. A. Zakian, PLoS Comput. Biol. 2010, 6, e1000861.
[19] P. Rawal, V. B. R. Kummarsarsetti, J. Ravindran, N. Kumar, K. Halder, R. Sharma, M. Mukerji, S. K. Das, S. Chowdhury, Genome Res. 2006, 16, 644–655.
[20] V. K. Yadav, J. K. Abraham, P. Mani, R. Kulshrestha, S. Chowdhury, Nucleic Acids Res. 2007, 36, D381–D385.
[21] M. L. Bochman, K. Paeschke, V. A. Zakian, Nature Reviews: Genetics 2012, 13, 770–780.
[22] M. Naizels, L. T. Gray, Plos Genet. 2013, 9, e1003468.
[23] O. Mendoza, A. Bourdoncle, J. B. Boule, R. M. Brosh, J. L. Mergny, Nucleic Acids Res. 2016, 44, 1989–2006.
[24] N. Naizels, Embo Rep. 2015, 16, 910–922.
[25] S. Balasubramanian, L. H. Hurley, S. Neidle, Nat. Rev. Drug Discovery 2011, 10, 261–275.
[26] H. Xu, M. Di Antonio, S. McKinney, V. Matthew, B. Ho, N. J. O’Neill, N. Dos Santos, J. Silvester, V. Wei, J. Garcia, F. Kabeer, D. Lai, P. Soriano, J. Banath, D. S. Chiou, D. Yap, D. D. Le, F. B. Ye, A. N. Zhang, K. Chu, J. Soong, S. C. Lin, A. H. C. Tsai, T. Osako, T. Alagara, D. N. Saunders, J. Wong, J. Xian, M. B. Bally, J. D. Brenton, G. W. Brown, S. P. Shah, D. Crescen, T. W. Mak, C. Palas, P. C. Stirling, P. Hieter, S. Balasubramanian, S. Aparicio, Nat. Commun. 2017, 8, 14432.
[27] R. Simone, P. Fratta, S. Neidle, G. N. Parkinson, M. A. Isaacs, FEBS Lett. 2015, 589, 1653–1668.
[28] S. Pelegarisa, M. Khan, G. Evan, Nat. Rev. Cancer 2002, 2, 764–776.
[29] T. Simonsson, P. Pecinka, M. Kubista, Nucleic Acids Res. 1998, 26, 3167–3172.
[30] U. Siebenlist, L. Hennighausen, J. Battey, P. Leder, Cell 1984, 37, 381–391.
[31] D. Z. Yang, L. H. Hurley, Nucleos. Nucleot. Nucl. 2006, 25, 951–968.
[32] A. Ambrus, D. Chen, J. X. Dai, R. A. Jones, D. Z. Yang, Biochemistry 2005, 44, 2048–2058.
[33] A. T. Phan, V. Kuryavyi, H. Y. Gaw, D. J. Patel, Nat. Chem. Biol. 2005, 1, 167–173.
[34] A. De Cian, E. DeLemos, J. L. Mergny, M. P. Teulade-Fichou, D. Monchaud, J. Am. Chem. Soc. 2007, 129, 1856.
[35] M. Livendahl, J. Jamroskovic, B. Prasad, J. S. Santos, J. Silvester, V. Wei, J. Garcia, F. Kabeer, D. Lai, P. Soriano, J. Banath, D. S. Chiou, D. Yap, D. D. Le, F. B. Ye, A. N. Zhang, K. Chu, J. Soong, S. C. Lin, A. H. C. Tsai, T. Osako, T. Alagara, D. N. Saunders, J. Wong, J. Xian, M. B. Bally, J. D. Brenton, G. W. Brown, S. P. Shah, D. Crescen, T. W. Mak, C. Palas, P. C. Stirling, P. Hieter, S. Balasubramanian, S. Aparicio, Nat. Commun. 2017, 8, 14432.