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Guanine Amine Derivatives Promote Forming a Base-Vacancy-G Quadruplex Structure

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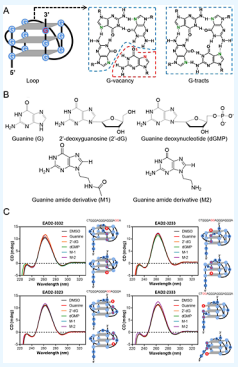
ABSTRACT: G-quadruplex (G4) structures are important in both biological and medical fields. A unique G4 type containing G vacancies allowing stabilization by guanine or guanine derivatives is widely used in the treatment of diseases, such as cancer. However, the identification of ligands with suitable affinities and specificities for G4 structures remains challenging. This study investigated the assisting effect of guanine derivatives in the formation of G-vacancy G4 structures. Focusing on the EAD2 sequence, we designed EAD2 sequences with one or two missing G bases. Circular dichroism (CD) assays revealed negligible impact of guanine and its derivatives on intact EAD2 structures but notable alterations in single-G-base-deficient EAD2 sequences. The formation of G4 structures in the EAD2 sequence lacking two G bases was difficult, even with the assistance of guanine derivatives. Moreover, experimental results demonstrated that the guanine derivatives **M2** had better auxiliary effects on base-deficient sequences than other compounds, which may be due to the enhanced pairing capability of G4 caused by **M2**. Lastly, the experiment demonstrates that the hemin/G4 DNAzyme-catalyzed ABTS confirmed the auxiliary role of the guanine derivatives in the formation of G4 in G base-deficient sequences. Overall, the findings indicated the significant impact of G base deficiency on G4 assembly, with the guanine derivative **M2** effectively aiding stable G4 formation in G base-deficient sequences.



1. INTRODUCTION

The G-quadruplex (G4) structure is formed specifically by DNA or RNA molecules and represents a planar structure formed by four guanine bases linked via hydrogen bonds.[1,2] This structure is widespread in the genome and plays an important role in biological processes such as gene regulation, the protection of chromosome terminals, and DNA replication and repair.[3−5] Despite extensive research elucidating the formation, biological functions, and potential applications of G4 structures, their stability and dynamic regulation remain hot topics in research.

Previous studies have shown that the formation of G4 structures is influenced by sequence specificity.[6,7] Certain nucleic acid sequences have a stronger tendency to form G4 structures, which provides important clues for further exploration of the mechanisms underlying the formation and biological functions of G4 structures.[8,9] However, the process by which different sequences form G4 structures, as well as the factors that influence this, still requires further investigation. Recent studies suggest that guanine derivatives may promote G-vacancy-containing sequences forming G4 structure, thus affecting the structure and function of DNA.[10−12] However, the underlying mechanisms and effects of different guanine derivatives on different G-vacancy sequences are not well understood. Therefore, a systematic study of the roles of guanine derivatives in G4 structure formation of G-vacancy-containing sequences can contribute to a deeper understanding of both the formation and overall stability of G4 structures.

This study aimed to explore the influence of different G-vacancy-containing sequences on G4 structure formation and the role of guanine derivatives in this process (Figure 1). We selected DNA sequences with different base compositions and designed variant sequences lacking one or two bases. Using circular dichroism (CD) spectroscopy, we analyzed the structures of these sequences under different conditions and evaluated the influence of guanine derivatives on G4 structure formation. This study first assessed the complete sequences in G4 structures to determine their ability to form stable structures without the addition of compounds. Subsequently, we examined sequences lacking one or two G bases, observing varying degrees of structural changes. Additionally, a guanine derivative, **M2**, that promotes G4 structure formation with sequences lacking one base was identified, and its efficacy surpassed other reported compounds.

2. MATERIALS AND METHODS

2.1. Compound Synthesis. *2.1.1. (2-(2-Amino-6-chloro-9H-purin-9-yl) Ethyl) tert-Butyl Carbamate (2).* (2-bromoethyl)*tert*-butyl carbamate (4.2 g, 15 mmol, 95%, Energy

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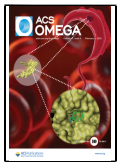

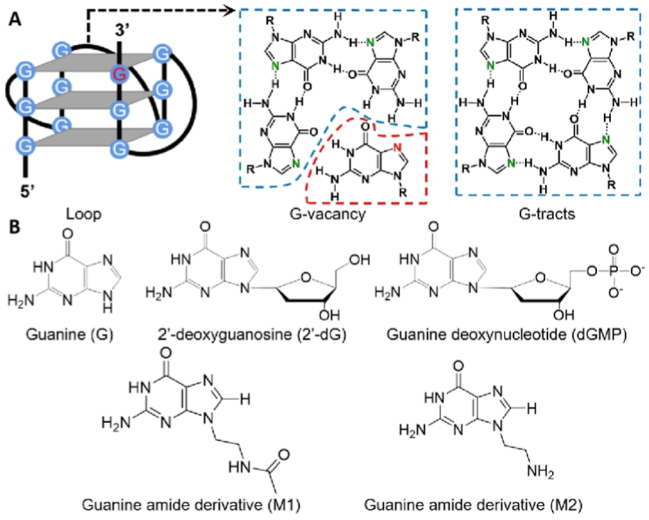

Figure 1. (A) Schematic diagram of the guanine-vacancy-G quadruplex and (B) structural formula of guanine and its derivatives: Guanine (G), 2′-deoxyguanosine, Guanine deoxynucleotide, Guanine amide derivative (**M1**), and Guanine amide derivative (**M2**).

Chemical) was added to a solution containing 2-amino-6-chloropurine (**compound 1**, 2.5 g, 15 mmol, 99%, Energy Chemical) and K_2CO_3 (5.0 g, 36 mmol, 99%, Energy Chemical) in dimethylformamide (DMF, 120 mL, Molecular Biology grade, Sangon Biotech). The resulting mixture was allowed to react at a temperature of 80 °C for 8 h. After completion of the reaction, the solvent was evaporated under reduced pressure, and the crude mixture was purified by flash silica gel column chromatography [eluent, ethyl acetate/petroleum ether (V/V) = 4/1] to yield **compound 2** (1.6 g) as a white solid. Yield: 34.2%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.98 (s, 1H), 6.96 (s, 1H), 6.86 (s, 2H), 4.08 (s, 2H), 3.32 (s, 2H), 1.32 (s, 9H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.15, 156.02, 154.73, 149.59, 143.81, 123.93, 78.36, 43.55, 28.58 ppm; MS (ESI) calcd for $C_{12}H_{17}ClN_6O_2$; $[M + H]^+$, 313.7660; found, 313.80.

2.1.2. 2-Amino-9-(2-aminoethyl)-1,9-dihydro-6H-purin-6-one (3). Intermediate **compound 2** (0.8 g, 2.6 mmol) was dissolved in 10 mL of 50% TFA/H_2O (99%, Energy Chemical) under nitrogen protection and stirred overnight. After completion of the reaction, the solvent was evaporated under reduced pressure, and the crude product was dissolved in methanol and precipitated by the addition of diethyl ether. Filtration purification yielded target **compound 3** (**M2**, 479.6 mg) as a white powder. Yield: 95%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.96 (s, 1H), 8.20 (s, 3H), 7.87 (s, 1H), 6.74 (s, 2H), 4.25 (t, J = 5.7 Hz, 2H), 3.29 (s, 2H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 156.75, 154.44, 151.46, 137.97, 115.97, 41.78, 38.61 ppm; MS (ESI) calcd for $C_7H_{10}N_6O$; $[M + H]^+$, 195.2060; found, 195.17.

2.1.3. N-(2-(2-Amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-ethyl)acetamide (4). **Compound 3** (291.3 mg, 1.5 mmol) was dissolved in DMF (15 mL, Molecular Biology grade, Sangon Biotech), followed by the addition of triethylamine (416.9 μL, 3.0 mmol, BC grade, Sangon Biotech) and acetic anhydride (422.6 μL, 4.5 mmol, AR, Guangzhou Chemical Reagent Factory) at room temperature and incubation for 6 h. After completion of the reaction, the solvent was evaporated under reduced pressure, and the residue was purified by washing with dichloromethane (200 mL, AR, Aladdin). **Compound 4** (**M1**, 180.7 mg) was obtained as a white solid. Yield: 51%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.57 (s, 1H), 7.97 (s, 1H), 7.60 (s, 1H), 6.45 (s, 2H), 3.99 (t, J = 5.2 Hz, 2H), 3.36 (d, J = 5.4 Hz, 2H), 1.78 (s, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ 170.08, 157.31, 153.93, 151.71, 138.06, 117.08, 42.87, 38.89, 22.96 ppm; MS (ESI) calcd for $C_9H_{12}N_6O_2$ $[M + H]^+$, 237.2430; found, 237.52.

Figure S1 shows the synthetic routes of guanine derivative **compound 3** (**M2**) and **compound 4** (**M1**). The ^{1}H NMR and ^{13}C NMR spectra of **compound 2**, **compound 3** (**M2**), and **compound 4** (**M1**) in the DMSO-d_6 solution are shown in Figures S2−S7.

2.2. Formation of G4 Structures. All DNA was purchased from Sangon Biotech (Shanghai). The DNA was dissolved in ultrapure water and quantified precisely using an Epoch 2 microplate spectrophotometer (BioTek) based on the absorbance values at OD_{260}. A 50 μM DNA solution was prepared in a G4/hemin catalytic reaction buffer and placed in a PCR instrument (T100, Bio-Rad). After heating at 95 °C for 5 min, the solution was rapidly cooled on ice for 10 min, followed by incubation at room temperature for 2 h to facilitate the formation of stable G4 structures.

2.3. Precise Quantification of Hemin. Suitable quantities of both the native hemin purchased from Alfa Aesar and modified hemin synthesized and purified by high-performance liquid chromatography were weighed and dissolved in dimethyl sulfoxide (DMSO, reagent grade, Sangon Biotech). A stock solution of approximately 10 mM concentration was prepared and tested for absorbance at 385 nm in a 0.1 M NaOH solution (BC grade, Sangon Biotech). After dilution to absorbance values within the range of 0.2 to 0.8, the exact concentration of each hemin solution was calculated using a molar absorption coefficient of 58,400 $cm^{-1}\cdot(mol/L)^{-1}$.

2.4. Measurement of hG4 DNAzyme Catalytic Activity. A mixture of 200 nM DNA G4 sequences and 300 nM hemin (AR, Alfa Aesar) was incubated at room temperature for 30 min in G4/hemin catalytic reaction buffer (20 mM MES with pH 6.0, 50 mM KCl, 1% DMSO). Then, 2 mM 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS, reagent grade, Sangon Biotech) was added, followed by the addition of 2 mM H_2O_2 (USP grade, Aladdin), and immediate measurement commenced; absorbance at 414 nm was monitored. The reaction rate of peroxidase activity was obtained by linear fitting with the increase of light absorption value in the first 30 s using GraphPad Prism software. Each data set was measured in triplicate at least.

2.5. Calculation of Binding Constants. The binding affinity between DNA G4 and hemin was determined by measuring the absorbance change at the Soret band (404 nm) of the solution. The dissociation constant (K_d) was calculated by fitting the titration curve by using the following equation

$$[\mathrm{DNA}] = K_d A/(A_\infty - A) + [H]_0 A/A_\infty$$

where [DNA] is the concentration of DNA G4; $[H]_0$ is the initial concentration of hemin; A is the change in absorbance at 404 nm for different concentrations of hemin; A_∞ represents the maximum change in absorbance at saturated DNA concentration.

2.6. CD Spectroscopy. KH_2PO_4/K_2HPO_4 (20 mM, pH 7.4) (Molecular Biology grade, Sangon Biotech), KCl (100 mM) (Molecular Biology grade, Sangon Biotech), and the G4 DNA strand (20 μM) were then added, after which 1% DMSO (Reagent grade, Sangon Biotech), guanine (Reagent grade,

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Sangon Biotech), 2′-deoxyguanosine (2′-dG) (For Signal Pathway Regulation, Sangon Biotech), and deoxyguanosine monophosphate (dGMP) (Molecular Biology grade, Sangon Biotech), as well as the guanine derivatives **M1**(**Compound 4**) and **M2** (**Compound 3**), were added separately. After 2 h of incubation, CD spectra were measured in a total volume of 300 μL using a 1 cm path-length cuvette with a slit width of 6 mm and a nitrogen flow rate of 2 mL/min. The CD spectra of G4 DNA sequences and the G4 DNA sequences with added guanine derivatives were recorded in the range 220−350 nm using a blank containing buffer only.

3. RESULTS

The sequences of G4 DNA vary, and thus, the rate of ABTS catalysis under H_2O_2 conditions also differs after reacting with hemin. According to previous studies, the EAD2 sequence with the highest reaction rate was selected for investigation.[9] Based on this, various sequences with missing G bases were designed, as shown in Table S1. The gray shading in the sequences indicates regions capable of forming G4 structures, while the purple font indicates sequences with missing G bases. Four sequences, each with one missing G base were designed, namely, EAD2−3332/3323/3233/2333, together with two types of sequences with two missing G bases, namely, EAD2−1333/3133/3313/3331 and EAD2−2233/2323/2332/3223/3232/3322, totaling 14 sequences.

First, we conducted CD spectra of 15 sequences, including the control group EAD2 and 14 sequences with missing a G base. G4 DNA solutions were prepared following the experimental steps described in the materials and methods section. In this reaction system, the intact EAD2 sequence forms a stable parallel G4 structure. DMSO (1%), guanine, 2′-dG, dGMP, and the synthesized guanine derivative **M1** and guanine derivative **M2** were added separately. Testing began after the incubation at room temperature for 2 h. The graphs of each sequence with the compound (or the control group with DMSO) were compared to observe whether the addition of the compound caused a structural transition and the extent of such a transition.

The EAD2 sequence capable of forming complete G4 structures was then assessed with the results shown in Figure 2. Compared to the control group (1% DMSO), there was no change in the CD spectra of the five experimental groups with added guanine, 2′-dG, dGMP, and synthesized guanine derivatives **M1** and **M2**, respectively. This indicates that the EAD2 sequence itself had already formed a stable G4 structure,

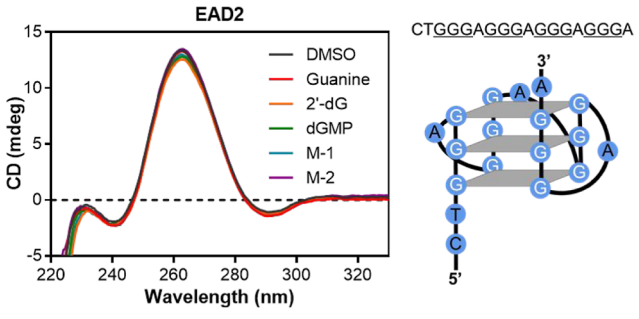

Figure 2. CD spectrum of the complete EAD2 sequence after adding each compound and the schematic diagram of the possible G4 structure.

and thus, the addition of guanine (or its derivatives) did not induce a structural change.

Figure 3 shows the CD spectra of EDA2 sequences with a missing G base, namely, EAD2−3332, EAD2−3323, EAD2−3233, and EAD2−2333. The CD spectrum of the DNA showed a positive peak at λ = 265 nm in 100 mm K^+, thus suggesting it formed a parallel G4.[13] Observing the CD spectra of each experiment, it was noted that, unlike the complete EAD2 sequence, there were slight alterations in the CD signal on the addition of the five guanine derivatives rather than overlap with the control group. This indicated that G base-deficient EAD2 affected the ability to form a complete G4 structure. The addition of guanine derivatives resulted in slight, but not significant, structural changes. Even with the assistance of guanine or its derivatives, there was no guarantee that stable G4 structures could be formed for different sequences. Only when there was a significant enhancement in the signal near 263 nm following guanine derivative compound addition could it be concluded that there had been a notable structural change. With the two carboxylic groups in the original hemin cofactor, modifications of these two tails can be achieved through amine−carboxylic coupling to generate amide derivatives. These modified hemin structures removed two negatively charged carboxylic groups and introduced hydrophilic tails and figure out that introduction of a histamine tail to function as a strong hydrogen transferring group modification would be the best for promoting the enzymatic efficiency.[9] Compared with guanine, guanine amine derivatives (**M1** and **M2**) were synthesized in this study, which own more electrons because of amide and amino groups.

For sequence EAD2−13331, the signal values after the addition of compounds were consistently lower than those of the control group (Figure 4), indicating that this design was unable to form a stable G4 structure, even with the assistance of compounds. Figure 3A also includes a schematic diagram illustrating the theoretical formation of G4 structures with the assistance of compounds. Since the first and fourth sets of G_3 in the sequence lack a G base, for the formation of the G4 structure, the last G near the 3′-end needs to be close to the 5′-end, which makes it possible to form a G4 structure by compound-assisted stabilization of the upper and lower layers. However, the 3′-end is inherently flexible, with a considerable degree of freedom, and it is thus difficult for the structure to form.

For the other four EAD2 sequences lacking one G base, changes in the EAD2 sequence structure were observed following compound addition, albeit with variations in the degree of assistance rendered by the compounds depending on the EAD2 sequence, as detailed in Table S2. Sequences where no significant structural changes were observed in the CD spectra were considered to have provided no apparent assistance, while those associated with changes were classified as providing either weak or strong assistance according to the degree of signal amplification. It was observed that guanine, 2′-dG, and dGMP showed auxiliary effects on the G-base deficient G4 structures, which is consistent with literature reports.[13] Of note is that the synthesized guanine amine derivative (**M2**) exhibited a significant degree of assistance in all four sequences, surpassing the reported efficacy of the other three compounds, possibly due to enhancement of the pairing ability by the amine group of **M2**.

Given the observed significant amount of assistance provided by **M2** on EAD2 sequences with one missing G

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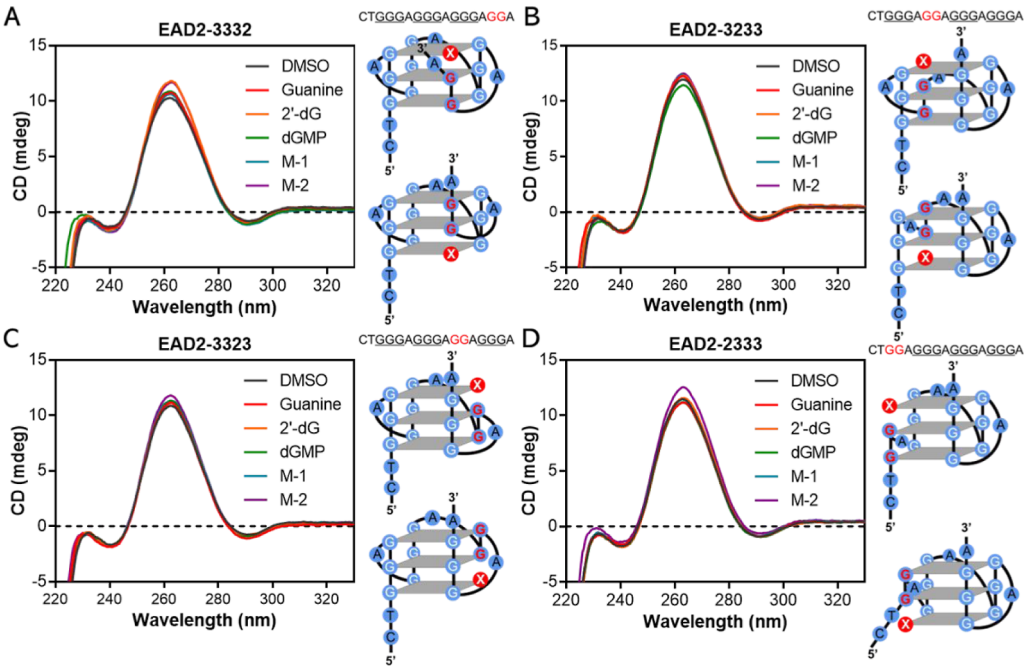

Figure 3. CD spectrum of the single base missing EAD2 sequence. (A) EAD2−3332, (B) EAD2−3233, (C) EAD2−3323, and (D) EAD2−2333 after adding each compound, and the schematic diagram of the possible G4 structure.

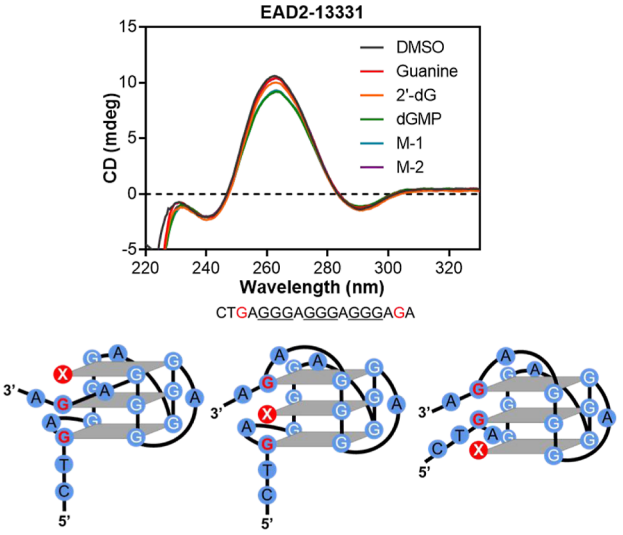

Figure 4. CD spectrum of the single base missing EAD2 sequence EAD2−13331 after adding each compound, and the schematic diagram of the possible G4 structure.

base, the effects of the two missing G bases were then investigated. To do this, two types of EAD2 sequence structures were designed, one with two missing G bases in one set of G_3, including sequences EAD2−1333, EAD2−3133, EAD2−3313, and EAD2−3331, and the other with one missing G base in each of two sets of G_3, including sequences EAD2−2233, EAD2−2323, EAD2−2332, EAD2−3223, EAD2−3232, and EAD2−3322.

The experimental results (Figure 5) indicated that for the EAD2 sequences with two missing G bases in one set of G_3, due to poor stability, only EAD2−3331 showed a slight structural change after the addition of compounds **M1** and **M2**, while the signals for the remaining three sequences after the addition of five compounds, as well as the control group, overlapped almost completely. This suggests that this type of G4 structure is difficult to form, even with the assistance of compounds.

For the six sequences with one missing G base in each of two sets of G_3, no structural changes in sequences EAD2−2233 (Figure 6A) and EAD2−2332 (Figure 6C) were induced by the addition of five individual compounds. For the other four sequences, compound **M2**, apart from showing a weaker effect on EAD2−3322 (Figure 6F), was found to have a significant impact on sequences EAD2−2323 (Figure 6B), EAD2−3223 (Figure 6D), and EAD2−3232 (Figure 6E). No notable phenomena were observed for the other compounds.

Based on these experimental results, we drew the following conclusions: (1) the formation of G4 structures was more difficult in the EAD2 sequence with two missing G bases when compared to those with one missing G base; (2) the positions of the two missing G bases significantly affected the formation of a complete G4 structure with the assistance of compounds; (3) the guanine derivative (**M2**) was able to bind more strongly than the amide derivative (**M1**) and guanine, 2′-dG, and dGMP. Therefore, even when other compounds could not act on G4 sequences with two missing G bases, the addition of **M2** still resulted in structural changes.

Several interesting phenomena can be observed from the schematic diagram (Figure 5) showing the formation of one representative structure from each of the six types of G4 sequences with two missing G bases. For the three sequences assisted by compound **M2**, the two sequences showing stronger effects were EAD2−2323 and EAD2−3232. This may be because the two chains with missing G bases were located at opposite positions, facilitating the formation of a complete G4 structure with the assistance of compound **M2**. In terms of sequence EAD2−3223, the weak assistance provided by the complete pairing of G_3 near the 5′- and 3′-ends reduced the flexibility of the chain. In terms of sequences EAD2−2233, EAD2−2332, and EAD2−3322, the chains with missing bases were adjacent, resulting in excessive flexibility and thus

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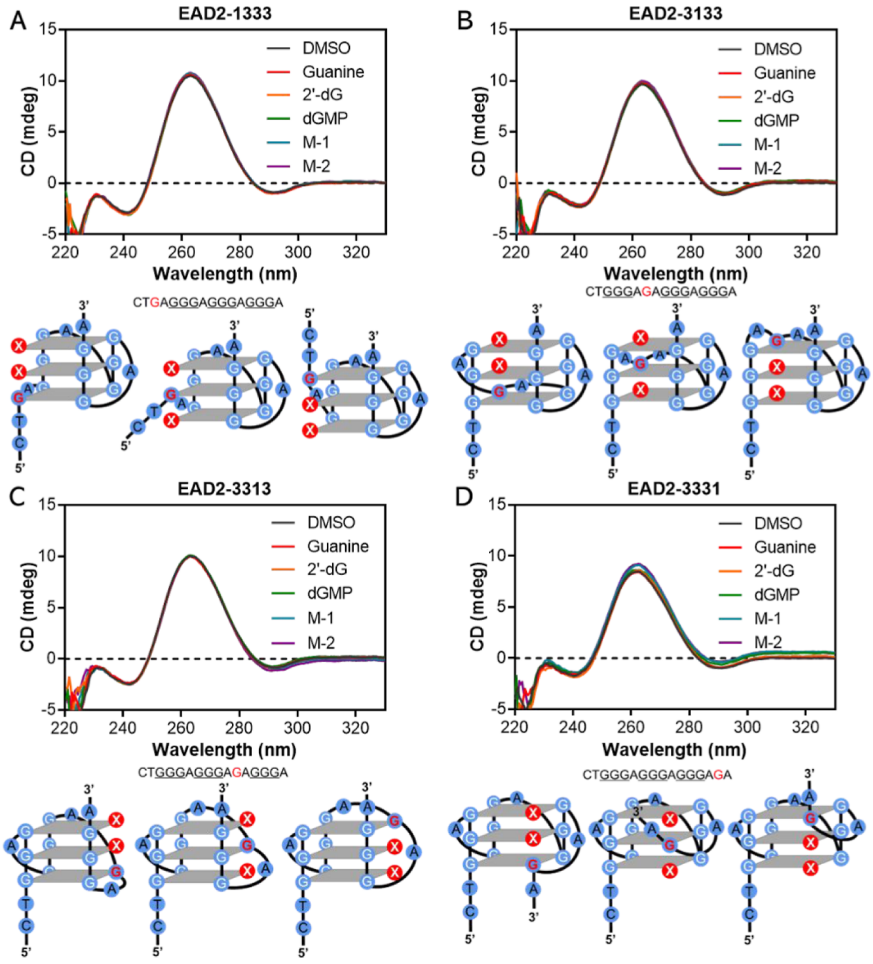

Figure 5. CD spectrum of the single base missing EAD2 sequence. (A) EAD2−1333, (B) EAD2−3113, (C) EAD2−3313, and (D) EAD2−3331 after adding each compound, and the schematic diagram of the possible G4 structure.

preventing the formation of a stable G4 structure, even with the assistance of compounds.

To further verify the formation of G4 structures after compound addition, the oxidation of ABTS by the hemin/G4 (hG4) DNAzyme in the presence of H_2O_2 was investigated. hG4 can simulate the catalytic activity of horseradish peroxidase and oxidize colorless ABTS to green $ABTS^+$.[9] The rate constants of 16 G4 DNA sequences and their respective additions of five compounds are listed in Table S3.

It can be seen that the original EAD2 sequence could form a complete G4 structure with the highest catalytic rate, with compound addition having no effect on the rate. The catalytic rates of the other 15 sequences with missing G bases were significantly reduced, and for some sequences, the addition of compounds led to a significant increase in their catalytic rates, corresponding roughly with the CD results. The most significant effect was observed for compound **M2** (the DMSO group serves as the control group, and the sequences showing significant rate increases are marked in red).

Furthermore, it was also observed that although compound **M2** had an enhancing effect on both sequences with one missing G base and those with two missing G bases, the effect was not significant, with 2- to 2.5-fold increases. The main reason for this is the difficulty in forming a G4 structure when a G base is missing; therefore, even with the assistance of compounds, the formation of an effective stable structure remains challenging. Conversely, some sequences may be in a metastable state after missing a G base, and thus compound addition can significantly stabilize the formation of G4 structures. However, the problem is that for catalytic reactions, the baseline before compound addition is too high, resulting in an insignificant change in catalytic efficiency after the addition of the assisting compounds, as can be seen from the relative reaction rates. Therefore, most baselines were above 0.2 (most were around 0.3). This indicates that even when the structure is in the most stable state, there is only a 3- to 5-fold enhancement after compound addition. The selected EAD2 sequence remained the G4 sequence with the highest reaction rate, consistent with previous reports.[9,14]

4. DISCUSSION

The results indicated that the reactions of different G4 DNA sequences with guanine derivatives differed. Specifically, the EAD2 sequence with the highest reaction rate was selected for investigation, and EAD2 sequences with missing G bases were designed. The results revealed that even with the assistance of guanine or its derivatives, stable G4 structures were not formed for all sequences. This indicates that the absence of bases had a significant effect on the formation of G4 structures.

The CD results also showed that in the EAD2 sequences lacking one G base, the degree of structural change was only slightly, but nonsignificantly, altered upon the addition of guanine or its derivatives. However, the formation of a

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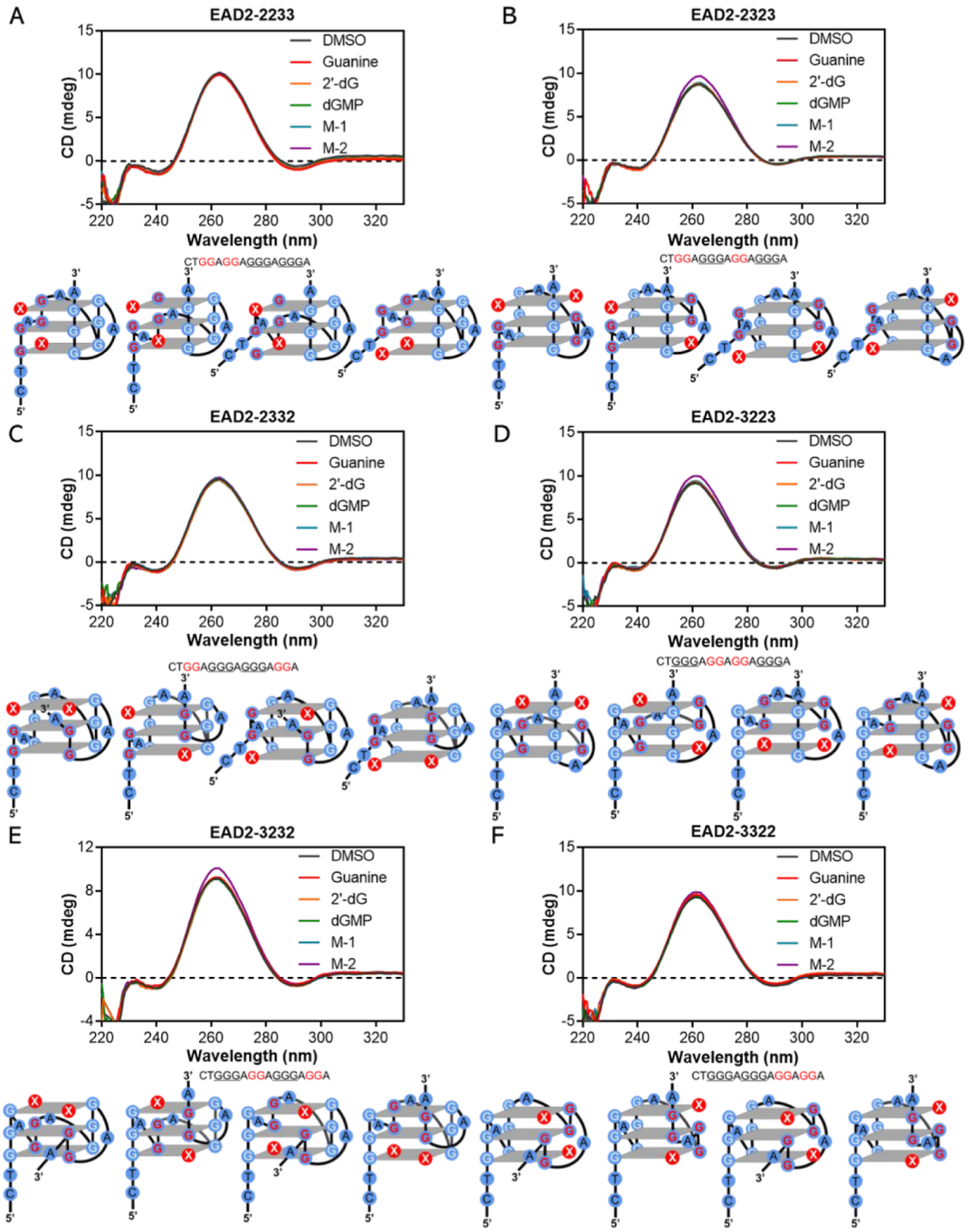

Figure 6. CD spectrum of the double bases missing EAD2 sequence. (A) EAD2−2233, (B) EAD2−2323, (C) EAD2−2332, (D) EAD2−3223, (E) EAD2−3232, and (F) EAD2−3332 after adding each compound, and the schematic diagram of the possible G4 structure.

complete G4 structure remained extremely challenging for sequences lacking two G bases, even with the assistance of the compounds. This suggests that the formation of G4 structures lacking two G bases is more difficult than those lacking only one base and that the position of the missing two G bases has a significant impact on whether the compounds can assist in the formation of a complete G4 structure. Interestingly, the effect of the guanine amine derivative (**M2**) was superior to that of other compounds, as reported by Xu's group, possibly due to the contribution of the **M2** amine group on pairing ability.[9] It is noteworthy that although **M2** offered significant assistance with sequences lacking one G base, it also induced structural changes in sequences lacking two G bases, albeit to a less noticeable extent. This indicates that even in the most stable state of structure formation, the assisting effect can be enhanced only to a limited extent.

Additionally, the formation of G4 structures after the addition of compounds was verified through hG4 DNAzyme experiments. The results showed a significant increase in the catalytic rate for certain sequences after the addition of compounds, consistent with the CD results. However, for some sequences, stable G4 structures were formed after compound addition. The change in catalytic efficiency was not significant, possibly due to the sequences themselves being in a substable state when bases were missing.

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In conclusion, the results of this study revealed the assisting effect of guanine derivatives on the formation of G4 structures with different missing G bases, providing important clues for a further understanding of these effects. We can further explore the potential value of these compounds in drug design and biomedical applications as well as their mechanisms of action at the cellular level.

5. CONCLUSIONS

The results of the promotion of base-deficient G4 structure formation by purine amine derivatives led to the following conclusions:

First, the formation of G4 structures lacking two G bases was more challenging than that when only one G base was missing. Even with the assistance of purine amine derivatives, only a few sequences lacking two G bases showed slight structural changes, while the signals in most sequences after compound addition overlapped almost completely with those of the control group. This indicates that this type of G4 structure is difficult to form even with the help of compounds.

Second, the position of the two missing G bases significantly affected whether the compound could assist in the formation of a complete G4 structure. Among sequences lacking two G bases, when the missing G bases were located at the end of G_3, some sequences showed only slight structural changes after compound addition.

Third, the purine amine derivative (**M2**) demonstrated a stronger binding ability than the amide derivative (**M1**) and purine, 2′-dG, and dGMP in promoting the formation of G4 structures. The experimental results of this study show that the synthesized purine amine derivative (**M2**) had a significant effect on sequences lacking one or two G bases, and its effect was superior to that of the other three compounds. This may be attributed to the presence of the amine group in **M2**, resulting in enhancement of the pairing capability.

In conclusion, guanine derivatives could assist in the formation of stable G4 structures from EAD2 chains lacking G bases. The amine-modified guanine derivative **M2** exhibited a higher propensity to form stable G4 structures with G-lacking EAD2 chains. Specifically, all four EAD2 chains lacking one G base could form more stable G4 structures upon the introduction of **M2**. Conversely, EAD2 chains lacking two G bases within the same G_3 region or lacking one G base in each of two adjacent G_3 regions showed difficulty in the formation of stable G4 structures, even in the presence of **M2**. However, EAD2 chains lacking one G base in each spaced G_3 region could form stable G4 structures upon the introduction of **M2**. Thus, **M2** provided a structural foundation for the formation of stable G4 structures in G-lacking EAD2. Our findings not only broaden the definition of sequences for G4 formation but also reveal a novel pathway for enriching the formation of stable G4 structures.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c09242.

Experimental synthetic routes, ^{1}H NMR spectrum of **compound 2** in the DMSO-d_6 solution, ^{13}C NMR spectrum of **compound 2** in the DMSO-d_6 solution, ^{1}H NMR spectrum of **M2** in the DMSO-d_6 solution, ^{13}C NMR spectrum of **M2** in the DMSO-d_6 solution, ^{1}H NMR spectrum of **M1** in the DMSO-d_6 solution, ^{13}C NMR spectrum of **M1** in the DMSO-d_6 solution, EAD2 and its guanine-vacancy sequence, ability of the compound to assist the four missing single base EAD2 sequences to form a stable G4 structure, and comparison of the catalytic rate of compound-assisted missing base EAD2 sequence to form a stable G4 structure (PDF)

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Author Contributions

#B.L. and J.Z. contributed equally. Y.L. designed the experiments, J.Z., B.L., H.Y., and J.W. carried out material synthesis and physicochemical characterization and drew the graphics, B.L., J.Z., and Y.L. wrote the draft of the manuscript. F.O. and Y.L. conceived the project and cowrote the paper. All authors have given approval to the final version of the manuscript. We thank Guangzhou Masscre Biotechnology Co., Ltd. for providing reagents and consumables.

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Notes

The authors declare no competing financial interest.

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