Meso-Substituted Thiazole Orange for Selective Fluorescence Detection to G-Quadruplex DNA and Molecular Docking Simulation

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1. INTRODUCTION

In recent years, much effort has been made to develop small molecular probes capable of selectively recognizing the DNA structure in therapeutic drug screening and biosensor construction since DNA molecules are not readily visible.1−4

G-quadruplex DNA (G-DNA) is a quadruple-stranded helical structure formed from guanine-rich DNA sequences and is dispersed in important genomic regions, such as telomeres, gene promoters, ribosomal DNA (rDNA), and mRNA.5−7 It has received increasing attention due to its significant biological functions in biological processes and therapeutic applications.8,9 Cyano family dyes such as thiazole orange (TO, including 2TO and 4TO) are widely used as a light-up fluorescent probe upon binding to almost all forms of DNA, but it exhibits poor selectivity for recognizing G-quadruplex DNA (G-DNA), which has significant biological functions in biological processes and therapeutic applications. Here, introducing benzyl substituent to the meso position of the methine chain of 2TO is expected to selectively recognize G-DNA. The spectroscopic titrations reveal that modified 2TO (meso-Bn-2TO) has almost no background fluorescence in solution and shows a preference to bind with G-DNA over ssDNA, dsDNA, and ct-DNA. Specifically, meso-Bn-2TO 1a displays a strong fluorescent signal upon interaction with G-DNA and a very weak fluorescent signal upon interaction with ssDNA, dsDNA, and ct-DNA, displaying considerable selectivity for G-DNA. However, parent 2TO all gives a fluorescent signal in G-DNA, dsDNA, and ct-DNA. The fluorescence intensity of 1a increases nearly 80−162 times when bound with different G-DNA.

The known meso-substituted thiazole orange analogues 1a−f were synthesized through the AlCl3-catalyzed reaction of the parent TO with benzyl alcohol derivatives according to the published procedure.10

2. RESULTS AND DISCUSSION

2.1. Synthesis. The known meso-substituted thiazole orange molecules (Scheme 1) with G-DNA and other forms of DNA, aimed at the research of small molecular probes having selective detection of G-DNA. As expected, the introduction of different benzyl substituents at the meso position of 2TO creates fluorescent signal discrimination toward certain G-DNA structures. Among the six known TO derivatives, 1a shows the better binding abilities to various G-DNA and other DNA structures. This result is also explained by the molecular docking method. Previous literatures indicate the interaction between TO, modified at the carbon or nitrogen of heterocycles,18−21 and various DNA, however, a few works are studied on methine chain modified TO acting on G-DNA.22,23

Received: July 25, 2020
Accepted: September 22, 2020
Published: October 2, 2020
2.2. Fluorescence Response of Dyes 1a–f toward Different DNA Substrates. The fluorescence response of 1a toward different kinds of DNA (Table 1) is recorded to investigate its selectivity for G-DNA. As displayed in Figure 1A, it shows preference to bind with G-DNA (bcl2, htg22, ckit1, telo21, c-myc, and pu18) over ssDNA (da21 and dt21), dsDNA (4at and ds26), and ct-DNA. Specifically, dye 1a displays almost no fluorescence in the Tris–HCl buffer solution, and ssDNA, dsDNA, and ct-DNA also give very weak fluorescent signal induction. However, dye 1a shows an enhanced fluorescent signal when G-DNA is added. Dye 2TO is used as the reference (Figure 1B). The fluorescence of 2TO is all increased upon the addition of dsDNA, G-DNA, or ct-DNA, which gives no selectivity for G-DNA.

From the interaction between different DNA substrates and dye 1a, the G-DNA htg22-induced fluorescence signal change is the biggest. In view of this, the interactions of htg22 with various meso-substituted 2TOs 1a–f are discussed. As shown in Figure 2, the intrinsic fluorescence of 1a–f is very weak. However, their fluorescence intensities are significantly enhanced when G-DNA is added. In the induced fluorescence signal changes, dye 1a is the best, and the remaining is in the following order: 1f > 1d > 1e, 1b, and 1c. These results demonstrate that 1a exhibits a much better ability to differentiate G-DNA from other forms of DNA than its analogues 1b–f.

2.3. Fluorescence Spectroscopic Properties of 1a Interaction with Different G-DNA Substrates. Owing to the good differential ability of 1a toward G-DNA, the binding property of 1a to different G-DNA substrates are also studied by fluorescence titrations. As shown in Figure 3A, 1a alone in the buffer displays an extremely weak fluorescent signal. With the gradual addition of G-DNA htg22, a significantly enhanced emission peak at approximately 550 nm appears and the signal increased gradually as the concentration of htg22 is increased in the solution. The significant fluorescence is also observed when 1a is treated with the G-DNA telo21, bcl2, ckit1, c-myc, and pu18 (Figure 3B). In detail, the fluorescence intensity increases nearly 140 times for c-myc and pu18 in comparison with that of 1a alone and ca. 80–100 times increase is found for bcl2, ckit1, and telo21. In particular, 162 times enhancement is obtained for htg22. The emission maxima could be over 550 nm, while c-myc represents the longest energy emission peaks near 578 nm with the most red-shifted at 57 nm.

With the data obtained via fluorescence titrations, the binding constants \( K_d \) and binding stoichiometric ratios \( n \) are

![Scheme 1](https://example.com/scheme1.png)

**Table 1. Sequences of Oligonucleotides Used in the Present Study**

| name | sequence | structure |
|------|----------|-----------|
| dt21 | TTTTTTTTTTTTTTTTTTTT | single-stranded DNA<sup>a</sup> |
| da21 | AAAAAAAAAAAAAAAAAAAAA | single-stranded DNA<sup>b</sup> |
| 4at | ATATATATATATATATAT | double-stranded DNA<sup>c</sup> |
| ds26 | CAATCGGATCGATCCGATTG | double-stranded DNA |
| bc2 | GGGCGGCGGAAGGCGGCGG | G-quadruplex DNA<sup>c</sup> |
| htg22 | AGGGTTAGGTTAGGTTAGG | G-quadruplex DNA |
| ckit1 | AGGGAGGCGCGCTGGGAGGG | G-quadruplex DNA |
| telo21 | GGGTATGATTAGGTTAGG | G-quadruplex DNA |
| c-myc | TGGGGAGGGTGGGGAGGG | G-quadruplex DNA |
| pu18 | AGGGTGGGGAGGGTGGG | G-quadruplex DNA |
| ct-DNA | | double-stranded DNA |

<sup>a</sup>Single-stranded DNA (ssDNA). <sup>b</sup>Double-stranded DNA (dsDNA). <sup>c</sup>G-quadruplex DNA (G-DNA).

Figure 1. Fluorescence intensities at \( \lambda_{em} (\lambda_{ex} = 488 \text{ nm}) \) of 1a (A) and 2TO (B) with different kinds of DNA in the Tris–HCl buffer containing 60 mM KCl. Concentration of dyes and DNA is all 10 \( \mu \text{M} \).
analyzed according to the independent site model by nonlinear fitting to eq 1.24 The results are summarized in Table 2. The binding constants of 1a and six G-DNA species are in the range of 0.13−3.16 μM. A comparison of the binding of dye 1a and 2TO (the binding constant of 2TO is 1.52 μM) with G-DNA shows that the binding affinity of dye 1a is much stronger. We believe that the introduced benzyl substituent at the meso position of 2TO shows a significant influence on the binding behavior of 1a, which can be further elaborated in the modeling study. In addition, the combination of 1a and htg22 is analyzed by the continuous variation method (Job plot) based on fluorescence emission spectroscopy (Figure 4). According to the coordinate of the intersection of the curves (0.67, 108.2), one htg22 is combined with two molecules 1a in the combination ratio of 2:1 (1a/G-DNA), which is consistent with the result of the above-described least-square simulation.

### Table 2. Fluorescence Titration Characteristics of 1a (10 μM) in Different G-DNA Substrates

| G-DNA | total DNA (μM) | λmax (nm) | λem (1a/G-DNA) (nm) | n | Kd (μM) |
|-------|----------------|-----------|----------------------|---|---------|
| htg22 | 15             | 521       | 550                  | 2 | 3.16    |
| telo21| 15             | 720       | 561                  | 2 | 1.77    |
| bcl2  | 15             | 720       | 570                  | 1 | 0.75    |
| ckit1 | 15             | 722       | 569                  | 2 | 1.00    |
| c-myc | 20             | 721       | 578                  | 1 | 0.13    |
| pu18  | 20             | 721       | 570                  | 1 | 0.21    |

Figure 2. (A) Fluorescence spectrum of dyes 1a–f with htg22 in a Tris−HCl buffer containing 60 mM KCl. (B) Fluorescence intensities at λem (λex = 488 nm) of dyes 1a–f with and without G-DNA htg22 in a Tris−HCl buffer containing 60 mM KCl. Concentration of dyes and G-DNA is all 10 μM.

Figure 3. (A) Fluorescence titration of 1a (10 μM) with the stepwise addition of the G-DNA htg22 (arrows: 0−3 molar equiv) in 10 mM of Tris−HCl buffer and 60 mM of KCl at a pH of 7.4. (B) Fluorescence intensity of 1a (10 μM) at λem with different concentrations of G-DNA at λex = 488 nm.

Figure 4. Job plot of dye 1a and htg22 in buffer solution. [1a] + [htg22] = 1.00 × 10−5 M.
time, the absorbance decreases by 32%, and the $\lambda_{\text{max}}$ shifts from 516 to 521 nm. The results suggest that there are end-stacking mode and groove binding mode in the binding of 1a to htg22.\textsuperscript{25} This is attributed to the fact that dye 1a is a nonplanar molecule with a cation.\textsuperscript{16}

2.5.2. Molecular Docking. The experimental results show that introducing a benzyl substituent onto the meso position of 2TO causes the modified dyes to give very good fluorescent signal discrimination to G-DNA, which is not the same as their parent 2TO, being nonselective for dsDNA and G-DNA (Figure 1A). To better understand the influence of meso-substituents on the fluorescent discrimination ability for G-DNA, molecular docking studies are performed to elucidate the molecular interactions of 2TO and 1a with G-DNA as well as dsDNA.\textsuperscript{26} First, the interaction of 2TO and 1a with htg22 is modeled by docking using the crystal structure of G-DNA htg22 (PDBID:143D) as templates. As shown in Figure 7, both 2TO and 1a stack on G-tetrad and exhibit a $\pi-\pi$ stacking interaction with htg22. For 1a, beyond the stacking interaction, the benzyl group of 1a pins to the outer wall of the G-DNA groove like a rake (Figure 7B), thus leading to high binding affinity. Being bound to G-DNA, the vibration or rotation of 1a is restricted. The results explain how dye 1a has a much

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Figure 5. CD spectra of 5 μM G-DNA: htg22 (A), telo21 (B), ckit1 (C), bcl2 (D), c-myc (E), and pu18 (F) in 10 mM Tris–HCl buffer, 60 mM KCl, pH 7.4, with and without 1a (0–20 μM).

Figure 6. UV−vis spectra titrations of htg22 stepwise to 1a (10 μM) in 10 mM Tris–HCl buffer, 60 mM KCl, pH 7.4.
stronger induced fluorescent signal upon binding to htg22 than that of 2TO.

Molecular docking study of 1a with duplex DNA is also conducted to explain its turn-on signaling preference toward the G-DNA structure rather than the duplex DNA (108D) (Figure 8), which is different from that of its parent scaffold 2TO. The docking model of 1a with duplex DNA shows that only the quinoline group is sandwiched in the 5′-CpT-3′ binding site, while the benzyl is perpendicular to the phosphate backbone and the benzothiazole group is likely to remain flexible in conformation, thus leading to a low binding affinity. In addition, the rotation of the methine bridge of 1a is not restricted by the crowding effect of the loops of phosphate backbone, resulting in very weak fluorescence enhancement. The parent 2TO prefers to stack planarly on the 5′-CpT-3′ binding site, acting as a duplex DNA intercalator, which agrees well with the molecular docking of the previous report. The docking study suggested that the benzyl chain incorporated into the meso position of 2TO enhanced the interactions with G-DNA but weakened the interactions with duplex DNA, which is consistent with the fluorescence spectra in solution.

2. CONCLUSIONS

The development of a series of sensitive nucleic acid probes (meso-Bn-2TO) based on meso-substituted thiazole orange framework is demonstrated. Among these dyes, 1a is chosen as the most promising candidate due to its highly selective fluorescence response to G-DNA. A molecular docking study on the interaction of 1a and 2TO with different DNA illustrates that the benzyl group incorporated into the meso position methine chain of 2TO enhances the interactions with G-DNA but weakens the interactions with duplex DNA, which is consistent with the fluorescence spectra in solution. The results indicate that 1a may be utilized for the selective detection performance of G-DNA.

4. EXPERIMENTAL SECTION

4.1. Materials. All solvents were obtained from commercial suppliers and used without further purification. 2TO and meso-Bn-2TO 1a−f were synthesized according to the published procedure. 17 Calf thymus DNA (ct-DNA) was purchased from Sigma Chemical Co. All oligonucleotides used in this work were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd (China), and the detailed sequences are listed in Table 1. The stock solutions of 2TO and meso-Bn-2TO 1a−f (1 mM) were prepared using dimethyl sulfoxide (DMSO) and diluted to the required concentration (1.0 × 10−5 M) with the Tris−HCl buffer solution (pH 7.4). The oligonucleotide was diluted individually with 10 mM Tris−HCl buffer solution (pH 7.4, containing 60 mM KCl) as the stock solutions (100 μM). G-DNA (bcl2, htg22, ckit1, telo21, c-myc, and pu18) stock solution was annealed to 95 °C for 10 min, followed by gradual cooling to room temperature and kept at 4 °C for overnight incubation. Duplex DNA (4at and ds26) stock solutions were prepared by heating the self-complementary strand at 85 °C for 5 min in the Tris−HCl buffer, followed by slow cooling over 6 h. The ct-DNA stock solution, which does not require annealing, was dissolved directly in the Tris−HCl buffer. The concentrations of duplex DNA and ct-DNA were estimated by measuring the UV absorbance at 260 nm (ε = 6600 M−1 cm−1) before use.

4.2. Spectroscopy Measurements. The absorption spectra were obtained using a Purkinje General TU-1900 UV−vis spectrometer using a 1 cm path length quartz cuvette. The fluorescence spectra were obtained using a PerkinElmer LS-55 fluorescence spectrophotometer. A quartz cuvette with a 2 mm × 10 mm path length was used for the spectra recorded at 10 and 5 nm for excitation and emission slit widths, respectively.

In the fluorescence titration experiment, DNA solution at the specified concentration was added to the 2TO or 1a−f solutions, and the resulting solution was allowed to incubate for 10 min before the fluorescence measurement was obtained at an excitation wavelength of 480 nm. The data from the spectral titrations were analyzed according to the independent site model by nonlinear fitting to eq 1. The parameters Q and n were found via the Levenberg−Marquardt fitting routine in the Origin 8.5 software, whereas n was varied to obtain a better fit.

\[
F/F_0 = 1 + \frac{Q - 1}{2} [A + 1 + x - \sqrt{(A + 1 + x)^2 - 4x}]
\]

(1)

where \(F_0\) is the fluorescence intensity of dye (maximum emission wavelength) in the absence of DNA and \(F_{\text{max}}\) is the fluorescence intensity upon saturation of DNA. Q = \(F_{\text{max}}/(F_0)^{-1}\), A = (\(K_C_{\text{DNA}}\))^−1, and \(x = nC_{\text{DNA}}(C_{\text{DNA}})^{-1}\).

4.3. Continuous Variation Analysis. Continuous variation analysis was performed on a PerkinElmer LS-55 fluorescence spectrophotometer. Stock solutions of 10 μM 1a and 10 μM G-DNA htg22 were prepared in the Tris−HCl buffer (10 mM, pH 7.4) containing 60 mM KCl. A series of solutions with varying mole fractions of 1a and htg22 were used for the experiments: the sum of 1a and htg22
concentrations was always 10 μM. Their fluorescence spectra were collected from 500 to 800 nm using a 2 mm × 10 mm path length quartz cuvette at 25 °C. The fluorescence emission intensity of these solutions at 555 nm was recorded, and then such difference in the fluorescence values at λem wavelength (555 nm) was plotted versus the ligand mole fraction to generate a Job plot. The final analysis of the data was carried out using Origin 8.5.

4.4. CD Measurements. The spectra were measured on a Chirascan circular dichroism spectrophotometer in the wavelength range 220–320 nm using a quartz cuvette with a 4.0 mm path length. The scanning speed of the instrument was set to 200 nm/s. The oligomer (htg22, bcl2, ckit1, telo21) was diluted from stock to the correct concentration (5 μM). CD titration was performed at a fixed concentration of DNA (5 μM) with the increased amount of meso-Bn-2TO 1a in the range 0–4 molar equiv at the condition of the Tris–HCl buffer (10 mM Tris–HCl, pH 7.4) with 60 mM KCl. The CD spectra were recorded after equilibrating for 10 min.

4.5. Molecular Docking. The G-DNA htg22 and a duplex DNA ds26 were used to perform a docking study on dye 1a and 2TO. The crystal structure of G4-DNA htg22 S'-AGGGTTAATGGTTAGGG-3' was collected from PDB under code 143D. The structure of duplex DNA S'-d(CGCTAGCG)-3' was collected from PDB under code 108D. The structures of dyes are optimized by the Gaussian 09 program (B3LYP/6-31G* level). The docking studies of DNAs with 1a or 2TO were performed using Dock 6.0 program and Lamarck genetic algorithm.29 The dimensions of the active site box were chosen to be large enough to encompass the entire G4 structure and the active site of duplex DNA. Each docking experiment includes 200 independent runs.

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Notes

The authors declare no competing financial interest.

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