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A far-red fluorescent probe for selective G-quadruplex DNA targeting

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ABSTRACT

The development of rapid and simple approaches for detection of G-quadruplex DNA structures has attracted significant attention to disclose their diverse physiological and pathological functions. Thiazole orange (**TO**) is a common fluorescence probe used for the detection of G-quadruplexes. However, it still suffers from some common problems like non-selective for G-quadruplex and emission in the lower wavelength region of spectrum, thus hampering its further applications. Probes with turn-on fluorescence in the far-red region are highly sought-after due to minimal auto-fluorescence and cellular damage. In this paper, we described a far-red fluorescent probe (**L-1**) by introducing an amine group into styrylquinolinium scaffold. The experimental results indicated that **L-1** exhibited significant fluorescence enhancement when treated with G-quadruplexes but retained weak fluorescence in the presence of duplex DNAs. In addition, this probe also displayed higher binding affinity for parallel G-quadruplexes. The characteristics of **L-1** were further investigated with UV–vis spectrophotometry, fluorescence, circular dichroism, KI quenching, FID assay and molecular docking to validate optical photophysical properties, as well as the selectivity, sensitivity and detailed binding mode toward G-quadruplex DNAs.

Single-stranded DNAs containing a repeating sequence of nucleobases can form alternative secondary structures through non-canonical base pairs. The most well-studied and highly characterized secondary structure in the past two decades is the G-quadruplex DNA, which is known to be formed by guanine-rich DNA sequences. The basic structural unit of G-quadruplex is the G-quartet, which is derived from the association of four guanines into a cyclic Hoogsteen hydrogen bonding arrangement [1–4]. Studies revealed that putative G-quadruplex-forming DNA sequences are highly prevalent in human genes, especially in telomere structures at the ends of chromosomes and in promoter regions of some oncogenes [5]. It is believed that G-quadruplex DNA structures play a significant regulatory role in many biological processes, such as transcriptional regulation, DNA replication, telomere maintenance and antitumor chemotherapy [6]. The ever-increasing interest in the area of the G-quadruplex requires the development of selective G-quadruplex-targeting probes to improve our understanding of G-quadruplexes and their binding characteristics [7].

Thiazole orange (**TO**) is an exceptional asymmetric cyanine dye. It possesses many distinctive and desirable properties: low intrinsic fluorescence emission, chemical stabilities, and a high molar absorption coefficient. Moreover, **TO** has a high binding affinity to double-stranded DNA with intense fluorescence enhancement [8]. These unique properties make **TO** particularly useful for the detection of double-stranded nucleic acids in a variety of techniques. In addition to duplex DNA, **TO** can also stack on G-quartet and has been reported for the detection of G-quadruplex DNAs [9]. The common problems like non-selective for G-quadruplex and emission in the lower wavelength region of spectrum (green region), hamper its further applications. In fact, probes with absorption in the longer wavelength of the visible region and emission in the far-red region (600–750 nm) are highly sought-after due to minimal auto-fluorescence and cellular damage [10]. These limitations need to be considered while designing new molecular probes. Several research groups have undertaken the challenging task of chemical modification of **TO** in order to enhance its binding affinity and selectivity and push its emission wavelength into the far-red region [11].

Based on our previous experience in designing selective G-quadruplex DNA fluorescent probes, we found that a flexible amine chain of the structure core was observed to be better in the terms

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of selectivity and binding affinity toward G-quadruplex DNA [12]. Herein, we attempted to design far-red fluorescent probes (**L-1** and **L-2**) by introducing flexible amine side groups to the **TO** template (Fig. 1), aiming to optimize the selectivity for G-quadruplex DNA and photophysical property. Their photophysical characterization and fluorescence performance on G-quadruplex DNA were investigated. **L-1** with a shorter amine chain gives a better selectivity for G-quadruplex DNA with far-red emitting. The detailed binding affinity and mechanism for G-quadruplex DNA was assessed by UV–vis spectrophotometry, fluorescence, circular dichroism, FID assay and KI quenching experiment. In addition, the binding mode of **L-1** was also studied by computational methods.

The synthesis of compounds **L-1** and **L-2** were achieved according to standard procedures. As shown in Scheme 1, The compound 4-methyl-1-(4-sulfobutyl)quinolonium (**1**) was prepared by alkylation reaction. Compounds **2** and **3** were synthesized by substitution reactions between 4-fluorobenzaldehyde and *N*, *N*, *N*'-trimethylethanediamine/*N*, *N*, *N*′-trimethylpropane-1, 3-diamine, respectively. The objective compounds were obtained by condensation reactions between compound (**1**) and (**2**)/(**3**). The compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS (see the Supplementary Information).

To evaluate the optical photophysical properties of the synthesized compounds, **L-1** and **L-2** were tested in different solvents (H_2O, DMSO, DMF, MeCN, EtOH, MeOH and DMK) by using an UV–Vis spectrophotometer and a fluorescence spectrophotometer (Fig. 2). Both of the two compounds showed absorption spectra in the range of 490–566 nm and displayed the shortest absorption wavelength in H_2O and the longest in DMSO. In protic solvents, the spectra exhibited a blue shift of the λ_{max} with increasing of the solvent polarity, while in the aprotic solvents the compounds underwent a red shift. From the emission spectra, the fluorescence intensity evidently decreased with the increasing solvent polarity and is the lowest in water. The possible reason is that the vinylic bond between the benzene and quinolonium was rotated in water which destroys the coplanarity of the molecule, thereby resulting in the increase of the nonradiative decay process and finally the obvious quenching occurred [13]. In addition, both of the compounds have large Stokes shifts in various solutions. The maximum values reached to 154 nm (for **L-1**) and 141 nm (for **L-2**) in water. Taken together, these spectral features are characteristic of internal charge transfer (ICT) effect upon excitation.

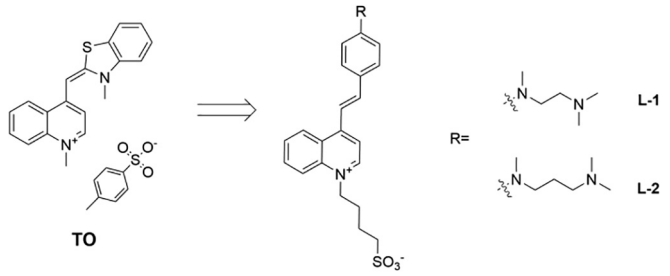
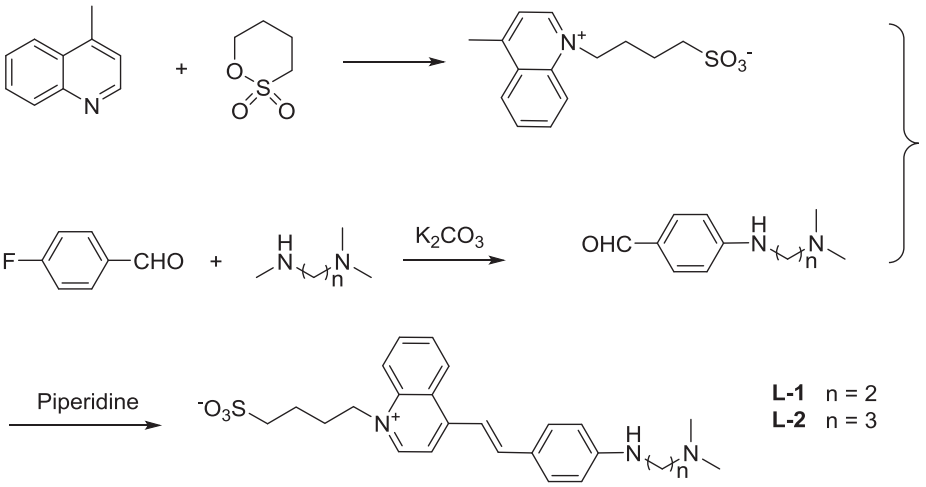


Fig. 1. Molecular structures of **L-1** and **L-2**.

The behaviors of **L-1** and **L-2** toward duplex and G-quadruplex DNA structures have been studied using fluorescence titration experiments to identify the most promising fluorescent probe. In these experiments, a fixed concentration of the compound (1 μM) was titrated against increasing concentrations of DNA. As shown in Fig. 3, with addition of DNA, the fluorescence intensity of the two compounds increased, suggesting there were certain interactions between DNA and compounds. The enhancements of fluorescence intensity of **L-1** were found to be remarkable upon binding with different G-quadruplex DNAs, while much smaller changes were observed with double-stranded DNAs. In contrast, under the same experimental conditions, the emission changes induced by **L-2** are markedly less significant than those observed with **L-1**. The results hinted that the shorter-chain fatty amine side chain in the scaffold of **L-1** was found to show high binding affinity and selectivity to G-quadruplex structures. For this reason, **L-1** was chosen for further detailed investigation.

The detailed interactions of **L-1** with DNA structures were further studied by means of fluorescence and UV/visible spectroscopy. The DNA solutions were added stepwise to a 1 μM solution of **L-1** in buffer and the fluorescence spectra were recorded after each addition. The titrations with all G-quadruplex DNAs resulted in a turn-on effect of the emission. Fig. 4a shows the emission spectra for the titration of compound **L-1** with *c-myc*. The fluorescence emission of **L-1** alone in buffer was very weak, with the gradual addition of the *c-myc*, an emission peak at approximately 640 nm was significantly enhanced. It is worth pointing out that the far-red region emission of **L-1** with G-quadruplex DNA was observed. The increase of the emission intensity depended on the added nucleic acid structure. When treated with other G-quadruplex DNAs (*22AG*, *Htg-21*, *CM22*, and *HRAS*) (Fig. S1), similar fluorescent turn-on property was also observed. In contrast, much smaller changes were observed when titrating **L-1** with duplex structures (*Ct-DNA*, *ds26*, *Polyd(A-T)*$_9$ and *Polyd(G-C)*$_9$). In order to examine



Scheme 1. Synthetic routes for the preparation of **L-1** and **L-2**.