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A simple structural modification to thiazole orange to improve the selective detection of G-quadruplexes

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ABSTRACT

Thiazole orange is a commonly used cyanine dye for binding to nucleic acids. Recently, it has been used for the detection of G-quadruplexes. However, thiazole orange is non-selective for G-quadruplex and other nucleic acids, thus hampering its further application. Herein, we designed and synthesized new fluorescent probes by incorporating hydrocarbon rings into the chromophore of thiazole orange. This simple modification dramatically improved selective binding to certain G-quadruplexes. The most promising probe, the cyclopentane fused analogue, exhibited significant fluorescence enhancement when treated with G-quadruplexes but retained weak fluorescence in the presence of double-stranded and single-stranded DNA. The cyclopentane fused probe also displayed considerable selectivity for parallel G-quadruplexes. These modifications reduced the quantum yield of thiazole orange. Further study of the mechanism revealed that the introduction of a hydrocarbon ring altered the planarity of the chromophore as well as the binding affinities for G-quadruplexes, and therefore, influenced the ability to detect G-quadruplexes.

1. Introduction

Thiazole orange (**TO**, Fig. 1) is an exceptional asymmetric cyanine dye. It is essentially non-fluorescent in aqueous solution and obtains intense fluorescence when bonded to double-stranded nucleic acids [1,2]. These unique properties make **TO** particularly useful for the detection of double-stranded nucleic acids in a variety of techniques. Examples include the detection of PCR products in real time, staining double-stranded DNA in agarose gels and capillary electrophoresis, and applications in fluorescent in situ hybridization [3–6].

In addition to double-stranded nucleic acids, **TO** is used for the detection of G-quadruplexes (G4s) [7,8]. G-quadruplexes are unique four-stranded structures that are formed by guanine-rich nucleic acid sequences. The building blocks of G-quadruplexes are G-quartets, which stack up on top of each other to form secondary DNA structures. G-quadruplexes are widely dispersed in eukaryotic genomes and can be divided into two main topologies: parallel and antiparallel structures [9–11]. During the past two decades, G-quadruplex structures have attracted considerable attention because of their biological significance and potential applications in supramolecular chemistry [12–14]. The ever-increasing interest in G-quadruplexes has promoted the development of rapid and simple approaches for the selective detection of these structures. Thus, the discovery of selective fluorescent probes for G-quadruplexes has become an extremely active area of research [15–25]. Notably, unlike the commercially available dye thioflavin T (ThT) which is a selective G-quadruplex probe [26–28], **TO** is non-selective for double-stranded and G-quadruplex nucleic acids. However, it may offer an attractive template for the design of selective fluorescent probes for G-quadruplexes. Successful examples include the bisquinolinium/**TO** conjugate and the benzofuroquinolinium/**TO** conjugate [29,30].

As shown in Fig. 1, the conjugates includes the assembly of the **TO** and the G-quadruplex binding small molecule all in a fusion scaffold with the aim of combining their advantages. These fluorescent probes exhibit distinct selectivity for G-quadruplexes over double-stranded nucleic acids. However, the conjugation strategy requires a suitable molecular framework to accommodate the **TO** chromophore. One relevant example is our previous work on the

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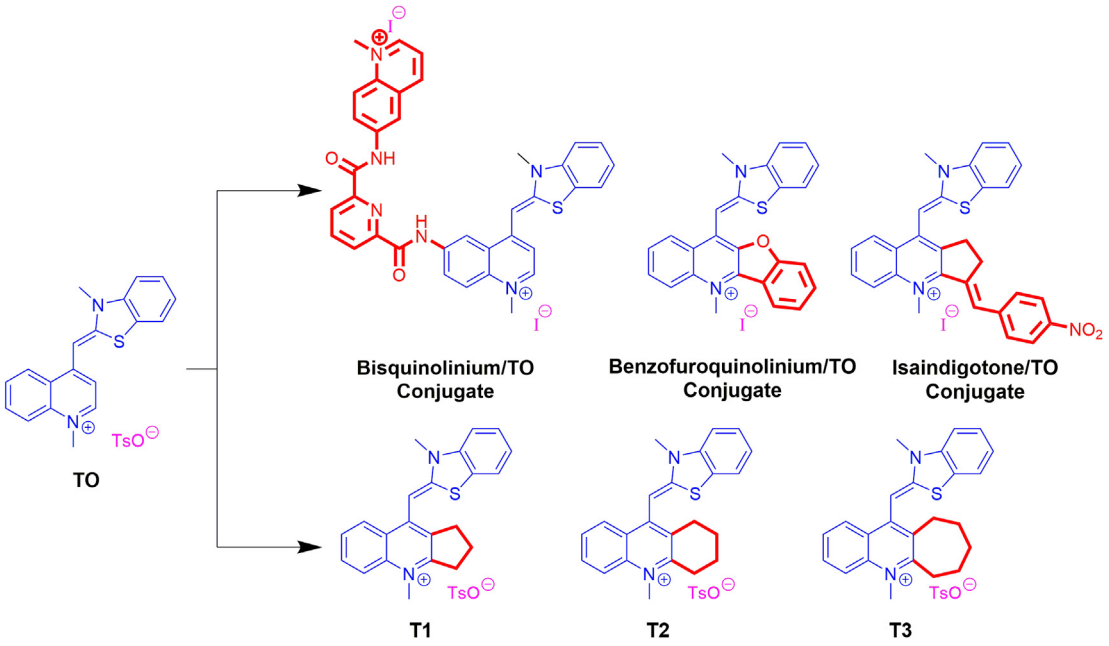

Fig. 1. Structures of **TO**, the bisquinolinium/**TO** conjugate, the benzofuroquinolinium/**TO** conjugate, the isaindigotone/**TO** conjugate and the **TO**-derived compounds: **T1**, **T2** and **T3**.

isaindigotone/**TO** conjugate [31]. This compound exhibits a high selectivity for the G-quadruplex, but it is non-fluorescent upon combination with the G-quadruplex. Incorporation of **TO** into the isaindigotone framework surprisingly eliminates the fluorescent properties of **TO**. Accordingly, it led us to reconsider the modification strategy, and we developed a G-quadruplex-specific fluorescent probe using a more simple structural modification to the **TO** scaffold.

Based on our experience in developing selective G-quadruplex binding ligands, we introduced some simple moieties to the **TO** scaffold [32–34]. Herein, we present a new series of **TO**-derived compounds: **T1**, **T2** and **T3**. Compared with **TO**, these compounds bear a hydrocarbon ring that has been suggested to be an important factor in the binding of small molecules to a G-quadruplex. Their photophysical properties were examined alone and in the presence of different nucleic acids. In addition, molecular modeling approaches were employed to assist in understanding the differences and the relevant mechanism.

2. Experimental methods

2.1. Synthesis and characterization

^{1}H and ^{13}C NMR spectra were recorded using TMS as the internal standard in $CDCl_3$ or DMSO-d_6 using a Bruker BioSpin GmbH spectrometer at 400 MHz and 101 MHz, respectively. IR spectra were determined on a Bruker Tensor 37 infrared spectrophotometer. Mass spectra (MS) were recorded on a Shimadzu LCMS-2010A instrument with an ESI or ACPI mass selective detector, and high resolution mass spectra (HRMS) were obtained on a Shimadzu LCMS-IT-TOF. Melting points (Mp) were determined using a SRS OptiMelt automated melting point instrument without correction. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. The purity of the synthesized compound was confirmed to be higher than 95% via analytical HPLC that was performed with a dual pump Shimadzu LC-20 AB system equipped with a Ultimate XB-C18 column (4.6 × 250 mm, 5 μm). All chemicals were purchased from commercial sources unless otherwise specified. All of the solvents were of analytical reagent grade and were used without further purification. The 2,3-dimethylbenzothiazolium tosylate was synthesized according to a previous report [35].

2.1.1. 9-Chloro-2,3-dihydro-1H-cyclopenta[b]quinolone (1)

To a stirred solution of 2-aminobenzoic acid (1.37 g, 10 mmol) and cyclopentanone (1.5 mL, 16.9 mmol) cooled in an ice bath, $POCl_3$ (11 mL) was carefully added and the mixture was refluxed for 5 h. After cooling to room temperature, the mixture was concentrated to give a slurry. The residue was then diluted with EtOAc, and neutralized with NaOH solution. The organic layer was dried over anhydrous $MgSO_4$. After concentration, the resulting residue was purified by flash chromatography with EtOAc: petroleum ether (1:3–1:5) to afford a white solid **1** (1.05 g, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (dd, J = 8.3, 1.0 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 7.72–7.66 (m, 1H), 7.61–7.55 (m, 1H), 3.26 (t, J = 7.7 Hz, 2H), 3.18 (t, J = 7.5 Hz, 2H), 2.31–2.22 (m, 2H). ESI-MS m/z: 204.0 $[M+H]^+$.

2.1.2. 9-Chloro-1,2,3,4-tetrahydroacridine (2)

The method for the preparation of compound **1** was used by replacing cyclopentanone with cyclohexanone. Compound **2** was synthesized as a white solid (1.07 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, J = 8.3 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.53 (t, J = 7.3 Hz, 1H), 3.13 (t, J = 5.7 Hz, 2H), 3.02 (t, J = 5.8 Hz, 2H), 2.05–1.91 (m, 4H). ESI-MS m/z: 218.1 $[M+H]^+$.

2.1.3. 11-Chloro-7,8,9,10-tetrahydro-6H-cyclohepta[b]quinoline (3)

The method for the preparation of compound **1** was used by replacing cyclopentanone with cycloheptanone. Compound **3** was synthesized as a white solid (1.12 g, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 8.2 Hz, 1H), 7.99 (d, J = 8.3 Hz, 1H), 7.67 (t, J = 7.1 Hz, 1H), 7.56 (t, J = 7.6 Hz, 1H), 3.31–3.18 (m, 4H), 1.95–1.87 (m, 2H), 1.86–1.71 (m, 4H). ESI-MS m/z: 232.1 $[M+H]^+$.

2.1.4. (Z)-4-methyl-9-((3-methylbenzo[d]thiazol-2(3H)-ylidene) methyl)-2,3-dihydro-1H-cyclopenta[b]quinolin-4-ium tosylate (*T1*)

A mixture of **1** (0.41 g, 2.0 mmol), methyl tosylate (0.9 mL, 6.0 mmol), and toluene (1 mL) was heated at 110 °C for 6 h. After cooling to room temperature, a solution containing 2,3-