Dyes and Pigments 137 (2017) 191–199

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Development of an engineered carbazole/thiazole orange conjugating probe for G-quadruplexes

Ming-Hao Hu [1], Rui-Jun Guo [1], Shuo-Bin Chen, Zhi-Shu Huang, Jia-Heng Tan*

School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, 510006, China

ARTICLE INFO

Article history:
Received 26 September 2016
Received in revised form
19 October 2016
Accepted 19 October 2016
Available online 19 October 2016

Keywords:
G-quadruplex
Carbazole derivative
Colorimetric probe
Fluorescent probe
Intracellular imaging

ABSTRACT

The development of selective and sensitive probes for sensing G-quadruplexes either *in vitro* or *in cellulo* has been the focus of investigation for a long time. Of those investigated, 3,6-bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC) is a promising fluorescent probe utilized in many studies investigating G-quadruplex structures. However, its shortcomings, including similar fluorescence responses for G-quadruplex and duplex DNA and green but not red fluorescent emission, might restrict the further application of BMVC. In this study, in order to improve the selectivity and optical properties of BMVC, we engineered a new probe (**TO-CZ**) by incorporating thiazole orange (TO) into the structure of BMVC. We next found that **TO-CZ** can act as a colorimetric and red-emitting fluorescent dual probe selective for G-quadruplexes without affecting the G-quadruplex topology. Further experiments showed that a 2:1 binding model involving the external binding of **TO-CZ** to both ends of the G-quadruplex is the most possible binding mode. Furthermore, we applied **TO-CZ** in sensing G-quadruplexes both *in vitro* and *in cellulo*, and found that **TO-CZ** can selectively and sensitively visualize G-quadruplexes. Notably, **TO-CZ** might be used to map DNA and RNA G-quadruplexes *in cellulo*, showing its great potential in investigating intracellular G-quadruplex structures.

1. Introduction

The development of highly selective and sensitive probes to detect nucleic acids is of profound importance to the fields of biology and clinical diagnostics [1]. G-quadruplexes are unique four-stranded structures formed by guanine-rich nucleic acid sequences. The basic structural unit of this structure is the G-quartet, which is derived from the association of four guanines into a cyclic Hoogsteen hydrogen-bonding arrangement [2]. G-quadruplex structures have been identified in the human genome with enrichment in telomeres, rDNA, promoter regions, untranslated regions of mRNA, and the first exons and first introns of many genes and are known to be highly associated with human diseases [3,4]. Accordingly, G-quadruplexes have captivated much attention because of their biological significance in recent years [5–7]. The ever-increasing interest in G-quadruplexes has resulted in the development of rapid and facile approaches for the sensitive and selective detection of these structures. Notably, G-quadruplex probes can be employed in the DNA-based sensing assays to detect specific analytes. Such functions further expand their scope of application [8,9]. Thus, visualizing G-quadruplexes using chemical sensors is an extremely active area of research, and significant progress has been made towards the development of colorimetric or fluorescent probes for G-quadruplexes [10–12].

Carbazole derivatives exhibit a wide range of pharmacological activities. Recently, a few carbazole derivatives have been reported to bind to G-quadruplexes [13–16]. Among them, 3,6-bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC) has attracted much attention. It is not only an effective G-quadruplex stabilizer but also a valid fluorescent probe of G-quadruplexes [17]. The fluorescent emission of BMVC is located at 570 nm upon interaction with G-quadruplex structures, while it is located at approximately 545 nm in the presence of duplex DNA. Thus, the different fluorescent colors of BMVC can be used to discriminate between duplex and G-quadruplex DNA [13]. Such a probe has been applied in many studies to reveal G-quadruplex structures either *in vitro* or *in cellulo* [18–24]. However, BMVC has very similar affinities for duplex and G-quadruplex DNA, showing similar fluorescence enhancements and emission bands upon binding to G-quadruplex and duplex DNA. This impedes the further application of BMVC, especially in

* Corresponding author.
E-mail address: tanjiah@mail.sysu.edu.cn (J.-H. Tan).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.dyepig.2016.10.022
0143-7208/

selective sensing G-quadruplexes in the presence of abundant double-stranded DNA both *in vitro* and *in cellulo*. Additionally, BMVC exhibits green rather than red emission, which might again restrict its *in cellulo* and *in vivo* imaging. It is notable that red-emitting fluorescent probes offer various advantages, including minimum photodamage to biological samples, diminished scattering of light, deep tissue penetration and minimum autofluorescence [25]. Thus, seeking a new carbazole probe possessing better properties, such as high selectivity and red emission, for G-quadruplex is important. Notably, one effective strategy for increasing the selectivity of a ligand for G-quadruplexes over duplex DNA is to expand its aromatic system by incorporating another aromatic moiety into the original molecule [26–30]. Such a modification might improve its fluorescence emission property when a fluorophore is introduced [29,30]. Therefore, we attempt to introduce a promising fluorophore into the BMVC scaffold. Such a conjugation strategy requires a suitable molecular framework to accommodate the BMVC scaffold. Therefore, we payed attention to thiazole orange (TO), because TO and BMVC share the same positive 1-methylpyridine moiety, making the two structures be easily fused into a new molecule. Notably, TO is one of the most widely used fluorescent probes in nucleic acid staining because of its high fluorescence quantum yield [31]. Although TO is non-selective for double-stranded and G-quadruplex nucleic acids, it offers an attractive template and serves as an outstanding fluorophore for the design of selective fluorescent probes for G-quadruplexes. Several probes for sensing G-quadruplexes have been discovered based on TO, with much better capabilities than their original molecules [26,28,32]. For all concerned, we are inspired to assemble BMVC and TO motifs into a fusion scaffold that is very convenient to be synthesized (Fig. 1). And we hope that such a modification would optimize the photophysical properties and selectivity of BMVC for G-quadruplexes.

In this study, a new carbazole/thiazole orange conjugate named **TO-CZ** was designed and synthesized. The detailed interactions of **TO-CZ** with various G-quadruplexes, double-stranded and single-stranded nucleic acids were investigated using UV–vis spectroscopic assays, fluorescence spectroscopic assays, circular dichroism spectroscopic assays and molecular modeling studies. **TO-CZ** was found to be a colorimetric and red-emitting fluorescent dual-output probe for G-quadruplexes with excellent selectivity. In addition, the detection abilities of the probe for G-quadruplexes were examined in both solution and a gel matrix. Furthermore, its potential application in cell imaging was explored.

2. Material and methods

2.1. Synthesis and characterization

2.1.1. General methods

^{1}H and ^{13}C NMR spectra were recorded using TMS as the internal standard in DMSO-d_6 with a Bruker BioSpin GmbH spectrometer at 500 MHz and 126 MHz, respectively. Mass spectra (MS) were recorded on a Shimadzu LCMS-2010A instrument with an ESI mass selective detector and high resolution mass spectra (HRMS) were recorded on Shimadzu LCMS-IT-TOF. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. The purity of synthesized compound was confirmed to be higher than 95% by using analytical HPLC with a dual pump Shimadzu LC-20 AB system equipped with a Ultimate XB-C18 column (4.6 × 250 mm, 5 μm), and eluted with methanol-water (80:20) containing 0.1% TFA at a flow rate of 1.0 mL/min. All chemicals were purchased from commercial sources unless otherwise specified. All the solvents were of analytical reagent grade and were used without further purification.

2.1.2. Synthesis of intermediates (*1*, *2* and *3*)

The intermediates (**1**, **2**, and **3**) were prepared using the methods following the procedures shown in Scheme 1 by our previous report [26,33].

2.1.3. Synthesis of (Z)-1,2-dimethyl-4-((3-methylbenzo[d]thiazol-2(3H)-ylidene)methyl)quinoli-nium iodide (*4*)

To a solution of **3** (0.54 g, 1.7 mmol) and 2,3-dimethylbenzothiazolium iodide (0.50 g, 1.7 mmol) in 40 mL methanol, sodium bicarbonate (0.30 g, 3.4 mmol), dissolved in 10 mL water was added. The reaction mixture was stirred at room temperature for 1 h and then reflux for 30 min. After cooling, aqueous solution of excess potassium iodide was added into the mixture. The precipitate was filtered, washed with water, acetone and dried under vacuum to afford the product as a dark brown solid **4** (0.61 g, 80%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.77 (d, J = 8.2 Hz, 1H), 8.18 (d, J = 8.8 Hz, 1H), 8.03–7.96 (m, 2H), 7.78–7.72 (m, 2H), 7.60 (t, J = 7.8 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 7.36 (s, 1H), 6.86 (s, 1H), 4.07 (s, 3H), 3.99 (s, 3H), 2.88 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.67, 154.77, 148.57, 141.06, 139.58, 133.63, 128.54, 126.87, 125.86, 124.73, 124.16, 123.97, 123.23, 118.79, 113.17, 111.11, 87.39, 37.56, 34.09, 23.23. ESI-MS m/z: 319.1 $[M - I]^+$.

2.1.4. Synthesis of 2-((E)-2-(9-ethyl-9H-carbazol-3-yl)vinyl)-1-methyl-4-((Z)-(3-methylbenzo[d]-thiazol-2(3H)-ylidene)methyl) quinolinium iodide (*TO-CZ*)

A mixture of **4** (0.45 g, 1.0 mmol), *N*-ethyl-3-carbazolecarboxaldehyde (0.25 g, 1.1 mmol) and 3 drops of piperidine in 4 mL of *n*-butanol was heated at 80 °C overnight. After cooling, the precipitate was filtered off, washed with *n*-butanol, and then purified on silica gel chromatography (CH_2Cl_2:CH_3OH = 50:1) to afford the dark red product **TO-CZ** (0.40 g, 61%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.72 (d, J = 8.1 Hz, 1H), 8.23 (d, J = 7.7 Hz, 1H), 8.16 (d, J = 8.7 Hz, 1H), 8.10–8.06 (m, 2H), 7.98–7.94 (m, 1H), 7.88 (d, J = 15.7 Hz, 1H), 7.76 (d, J = 15.8 Hz, 1H), 7.74–7.65 (m, 5H), 7.56 (t, J = 8.3 Hz, 1H), 7.52 (t, J = 7.2 Hz, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.28 (t, J = 7.4 Hz, 1H), 6.85 (s, 1H), 4.49 (q, J = 7.0 Hz, 2H), 4.20 (s, 3H), 3.96 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H). ^{13}C NMR

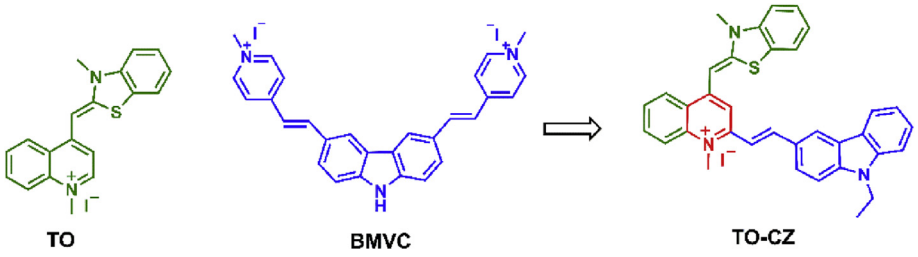

Fig. 1. The structures of thiazole orange (TO), BMVC, and **TO-CZ**.