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Original article

12-*N*-Methylated 5,6-dihydrobenzo[*c*]acridine derivatives: A new class of highly selective ligands for *c-myc* G-quadruplex DNA

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ABSTRACT

12-*N*-Methylated and non-methylated 5,6-dihydrobenzo[*c*]acridine derivatives were designed and synthesized as new series of *c-myc* G-quadruplex binding ligands. Their interactions with *c-myc* G-quadruplex were evaluated using fluorescence resonance energy transfer (FRET) melting assay, circular dichroism (CD) spectroscopy, surface plasmon resonance (SPR), polymerase chain reaction (PCR) stop assay, and molecular modeling. Compared with the non-methylated derivatives, 12-*N*-methylated derivatives had stronger binding affinity and stabilizing ability to *c-myc* G-quadruplex structure, and could more effectively stack on the G-quartet surface. All these derivatives had high selectivity for *c-myc* G-quadruplex DNA over duplex DNA. The reverse transcription (RT) PCR assay showed that compound **21c** could down-regulate transcription of *c-myc* gene in Ramos cell line containing NHE III_1 element, but had no effect in CA46 cell line with NHE III_1 element removed.

1. Introduction

Guanine-rich sequences can self-associate into planar guanine quartets (G-quartets) that stack on each other to form unusual structures called G-quadruplexes [1,2]. G-Quadruplexes can be stabilized by cations (preferring K^+) and widely distribute in telomeric regions [3,4] and gene promoters such as *c-myc* [5,6], *c-kit* [7], *bcl-2* [8], and *VEGF* [9]. The formation or stabilization of G-quadruplexes in these regions may result in a series of biological processes such as inhibition of telomerase [10], prevention of telomere elongation [11], avoidance of chromosomal alignment and recombination [3], and down-regulation of gene expression [12]. Ligands that can selectively bind to and stabilize G-quadruplex can interfere with telomere maintenance [13] or regulate gene expression [14,15], therefore are promising lead compounds for cancer treatment [16,17]. Several series of *c-myc* G-quadruplex ligands have been developed, including macrocyclic [18–20], large coplanar [14,18,20–22], and flexible molecules [20,23–25], in which some are alkaloid derivatives or their methylated products, and the latter showed better potency.

Acridine is a fused polycyclic aromatic molecule, which is a main scaffold in some natural acridine alkaloids [26]. Its derivatives such as amasacrine and quinacrine have been originally used as effective DNA-intercalating agents [27]. Recent years, acridine derivatives have been modified as telomeric [28–34] or *c-myc* [18,20] G-quadruplex ligands, in which some excellent ligands have been discovered, such as BRACO-19 [20], BOQ1 [32], and RHPS4 [35]. Although most of these ligands have shown high telomerase inhibitory activity ($IC_{50} < 1.0$ μM), they have exhibited moderate stabilizing ability ($\Delta T_m \sim 10$ °C) and low selectivity to G-quadruplex structures. It should be noted that an effective G-quadruplex binding ligand should have not only good bioactivity, but also high selectivity, in order to avoid acute toxicity and intolerable side effects in normal tissues.

With this aim in mind and based on the previous studies, we designed and synthesized a new class of crescent-shaped 5,6-dihydrobenzo[*c*]acridine derivatives (Fig. 1) as *c-myc* G-quadruplex ligands. Unlike the previous completely coplanar acridine molecules, our new acridine derivatives have a saturated C–C bond at the 5,6-position forming a slightly-twisted planar ring scaffold with non-methylated (Fig. 1A) and methylated (Fig. 1B) structures. The rationale for designing these derivatives are as follows: At first, it is interesting to know whether these partially-saturated acridine derivatives can act as G-quadruplex binding ligands, with good

Abbreviations: FRET, fluorescence resonance energy transfer; CD, circular dichroism; SPR, surface plasmon resonance; NMR, nuclear magnetic resonance; PCR, polymerase chain reaction; RT, reverse transcription; MTT, methyl thiazolyl tetrazolium.

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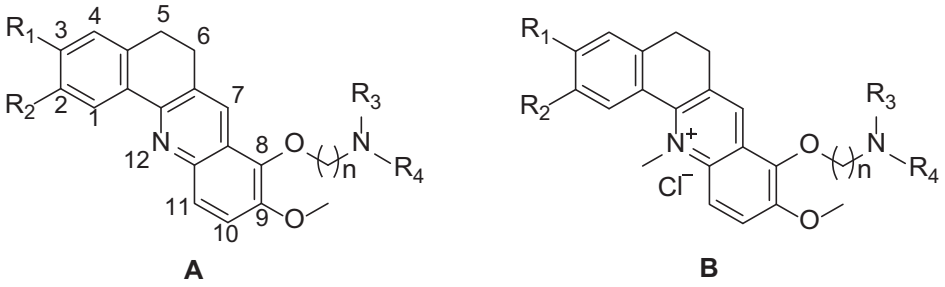


Fig. 1. Scaffold of 5,6-dihydrobenzo[*c*]acridine derivatives.

selectivity toward G-quadruplex DNA over duplex DNA. Secondly, some previous studies [35,36] have shown that the methylation of the nitrogen atom in the center of the ligands could strongly enhance their binding and stabilizing activity toward G-quadruplex, therefore, it is interesting to know whether the methylation of our new acridine derivatives can also increase their interactions with G-quadruplex. Thirdly, some studies have shown [32,37] that the fused crescent-shaped planar structure could offer effective π-orbital stacking on the G-quadruplex, therefore it is interesting to know whether our nearly planar crescent-shaped ligands can also effectively interact with G-quadruplex through π-orbital stacking. Lastly, it is interesting to know whether the 1,3-dioxole ring is better for the ligands to interact with G-quadruplex than the two methoxy groups. Therefore, we synthesized a series of 5,6-dihydrobenzo[*c*] acridine derivatives (Fig. 1), and studied their interactions with *c-myc* G-quadruplex DNA through FRET-melting, CD spectroscopy, SPR assay, PCR-stop assay, RT-PCR assay, and molecular modeling.

2. Chemistry

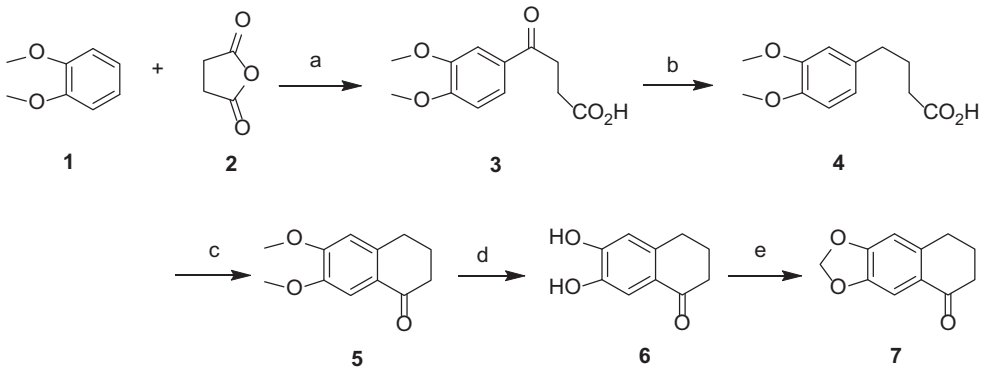
The 5,6-dihydrobenzo[*c*]acridine derivatives were synthesized as follows: 1,2-dimethoxybenzene **1** was acylated with dihydrofuran-2,5-dione **2** using $AlCl_3$ as catalyst in nitrobenzene at 0–60 °C to afford compound **3**, which was then deoxidized [38] and intra-cyclized to afford intermediate **5**. The intermediate **5** was demethylated with 48% HBr to give compound **6**, which was cyclized with dibromomethane under KF to yield intermediate **7** (Scheme 1) [39].

Compound **8** was synthesized by following a previously reported procedure [40], which then had the oxidation of its formyl group and the reduction of its nitro group to yield intermediate **9** (Scheme 2). Next the intermediate **5** or **7** was condensed with **9** in phosphorus oxychloride under reflux condition [41], and the product **10** or **11** had dechlorination, hydrolysis of sulfonic group, and alkylation with dibromoalkanes to afford compound **12**–**14**. Then the bromine atom was replaced with amine to yield final products **15a**, **15b**, **16a**, **17a**, and **17c**. Compound **12**–**14** could also be methylated with methyl triflate on the nitrogen atom [42], and then the bromine atom was replaced with amine, and lastly OTf_3^- was exchanged into Cl^- using anion exchange resin [43] to yield final products **20a**–**e**, **21b**, and **21c** (Scheme 2).

3. Result and discussion

3.1. Stabilizing ability and selectivity studies with FRET

The stabilizing ability of 5,6-dihydrobenzo[*c*]acridine derivatives on *c-myc* G-quadruplex DNA was evaluated with FRET-melting experiments [44]. The *c-myc* gene FPu18T (5′-FAM-$AG_3TG_4AG_3TG_4$-TAMRA-3′) was used as the G-quadruplex DNA model. The FRET-melting data (Table 1) showed that the ΔT_m values for all the compounds were in a wide range from 5.3 °C to 25.4 °C. The FRET results indicated that the methylated derivatives (ΔT_m values of 11.6–25.4 °C) had higher stabilizing ability than the non-methylated ones (ΔT_m values of 5.3–11.2 °C), which suggested that the central positive charge made an important contribution for the methylated derivatives to interact with *c-myc* G-quadruplex, and implied the nitrogen atom at the 12-*N*-position had weak tendency of protonation. Among all the methylated derivatives, **21b** and **21c** with the 2,3-dimethoxy moiety had the highest potency to stabilize the *c-myc* G-quadruplex DNA with ΔT_m values of 25.4 °C and 23.2 °C respectively, while their counterparts **20b** and **20c** with the 1,3-dioxole ring had lower affinity with the ΔT_m values of 22.2 °C and 21.4 °C, respectively, indicating the 1,3-dioxole ring was unfavorable for the interaction of 5,6-dihydrobenzo[*c*]acridine derivatives with G-quadruplex. Similar results were obtained for



Scheme 1. Synthesis of intermediates **5** and **7**. Reagents and conditions: (a) $AlCl_3$/nitrobenzene, 0–60 °C, 3 h; (b) Et_3SiH/CF_3CO_2H, reflux, 2 h; (c) PPA/DCM, reflux, 2 h; (d) 48% HBr, 125 °C, 4 h; (e) CH_2Br_2, KF/DMF, 140 °C, 6 h.