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Research paper

Design, synthesis and biological evaluation of 4-anilinoquinazoline derivatives as new *c-myc* G-quadruplex ligands

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ABSTRACT

A series of 4-anilinoquinazoline derivatives were designed and synthesized as novel *c-myc* promoter G-quadruplex binding ligands. Subsequent biophysical and biochemical evaluation demonstrated that the introduction of aniline group at 4-position of quinazoline ring and two side chains with terminal amino group improved their binding affinity and stabilizing ability to G-quadruplex DNA. RT-PCR assay and Western blot showed that compound **7a** could down-regulate transcription and expression of *c-myc* gene in Hela cells, which was consistent with the behavior of an effective G-quadruplex ligand targeting *c-myc* oncogene. More importantly, RTCA and colony formation assays indicated that **7a** obviously inhibited Hela cells proliferation, without influence on normal primary cultured mouse mesangial cells. Flow cytometric assays suggested that **7a** induced Hela cells to arrest in G0/G1 phase both in a time-dependent and dose-dependent manner.

1. Introduction

G-quadruplexes are nucleic acid secondary structures formed from guanine-rich sequences, and comprise a planar arrangement of four guanines (G-quartet, Fig. 1A) stabilized by Hoogsteen hydrogen bonding and monovalent cations [1]. Since G-quadruplex structures are widely located in plenty of important regulatory regions including telomeric DNA, oncogene promoters (such as *c-myc*, *VEGF*, *bcl-2*, *k-RAS*, *h-RAS*, *c-kit*, *HIF* and *HSP90*) and 5′-UTR, it is considered playing a significant role in regulating biological processes such as replication, translation and splicing [2,3]. The presence of G-quadruplex structures in human cells is now firmly established with antibody [4], which provides the basis for the elucidation of their function in normal and disease states.

c-MYC is a transcription factor whose expression is associated with cell proliferation. Increased levels of *c-myc* expression are observed in 80% of human cancer cells, and its increase promotes tumorigenesis [5]. The nuclear hypersensitivity element III_1 (NHE III_1), a guanine-rich strand of the DNA containing a 27 base pair sequence, which is upstream of *c-myc* promoter, controls 80–90% of the *c-myc* transcription [6]. The NHE III_1 can form intramolecular G-quadruplex structures and functions as a transcriptional repressor [7]. The transcription of *c-myc* can be down-regulated through stabilization of the G-quadruplexes by using specific G-quadruplex binders [8]. A number of *c-myc* G-quadruplex ligands have been reported, including phenanthroimidazole derivatives [9], acridine derivatives [10], prolinamide derivatives [11], platinum(II) complex [12], and showed pronounced antitumor activity.

Inspecting the structures of these G-quadruplex ligands, it is easy to find that most of them are based on a planar aromatic system that interacts with the G-quartet through π–π stacking interaction, with cationic side chains that interact with the negatively charged phosphate backbones in G-quadruplex [13,14]. For these molecules, optimal activity has been achieved through substitution of side chains with terminal amino group, especially with an amide bond conjugated with the aromatic ring system to maintain planarity on the edge of the G-quartet [15]. Besides, intensive research has suggested that extended aromatic surface always favor ligand-quadruplex binding interactions [16–18]. Neidle and co-workers have increased G-quadruplex binding affinity and biological activity of 3,6-disubstituted acridines by rationally adding an anilino subsituent at the 9-position to give 3,6,9-

Abbreviations: FRET, fluorescence resonance energy transfer; T_m, melting temperature; SPR, surface plasmon resonance; CD, circular dichroism; MTT, methyl thiazolyl tetrazolium; RT-PCR, reverse transcription-polymerase chain reaction; RTCA, real time cell analysis.

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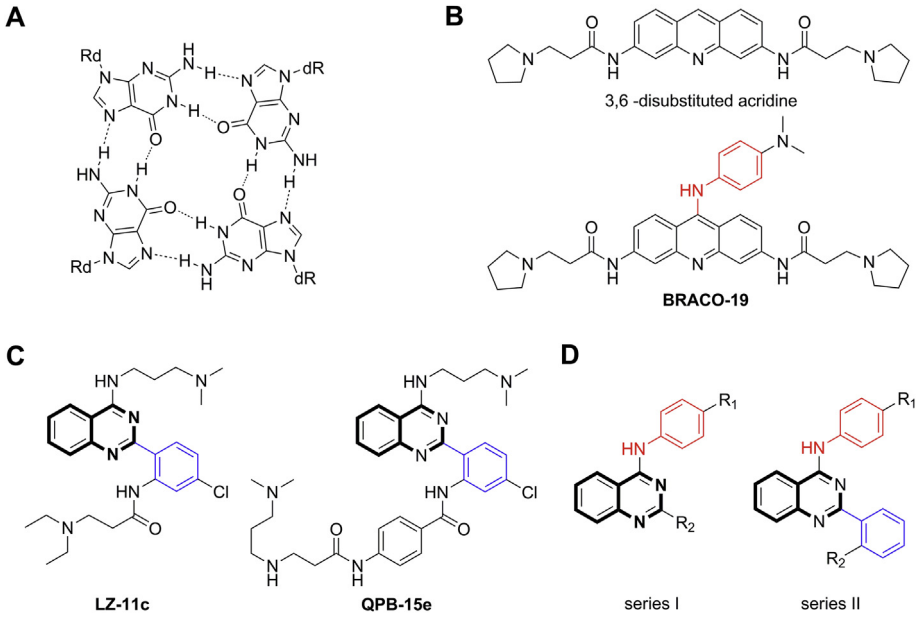

Fig. 1. Structures of (A) G-quartet, (B) 3,6-disubstituted acridine and **BRACO-19**, (C) 2,4-disubstituted quinazoline derivatives **LZ-11c** and **QPB-15e**, and (D) new 4-anilinoquinazoline derivatives (series I and II).

trisubstituted acridine molecules [19–21], one of which was **BRACO-19** (Fig. 1B).

Quinazoline derivatives, which have a planar chromophore, may interact with G-quartet of G-quadruplex DNA. In our recent study, a few 2,4-disubstituted quinazoline derivatives have been synthesized and found to be telomeric G-quadruplex binders, one of which is compound **LZ-11c** (Fig. 1C) [22]. Subsequently, a new substituted benzene ring has been linked to the *ortho*-position of 2-phenyl group of original quinazoline derivatives through amide bond, like compound **QPB-15e** (Fig. 1C), which have shown stronger binding ability and better selectivity for telomeric G-quadruplex [23]. In order to further develop new *c-myc* G-quadruplex binding ligands, we designed a series of new 4-anilinoquinazoline derivatives, in which aromatic system of the quinazoline is expanded by introducing an anilino group into the 4-position of quinazoline moiety for improving its G-quadruplex binding activity and stability. Unlike rigid aromatic compounds, such unfused aromatic molecules with adaptive structural feature could prevent themselves from intercalating into the duplex DNAs [6]. Based on above consideration, two series of derivatives were designed, including series I and II (Fig. 1D). For series I, we introduced an amide side chain with basic amino terminal at the *para*-position of aniline group (**1a**, **1b**, **2a**, and **2b**), which could be protonated at physiological pH, to establish additional electrostatic interaction with the target. Besides, we also replaced the amide bond with ether bond for the derivatives to uncover their influence on the G-quadruplex recognition (**4a** and **4b**). For Series II, a phenyl group was added at the 2-position of 4-anilinoquinazoline ring (**3a**–**b**, **5a**–**f**, **6a**–**d**, **7a**–**i**, **8a**, **8b**, and **9a**) to further extend the planarity of unfused aromatic system. In order to investigate the importance of two side chains on ligand-quadruplex interaction, the second amide side chain was appended to *ortho*-position of 2-phenyl group (**6a**–**d**, **7a**–**i**, **8a**, **8b**, and **9a**). Also it should be noted that the intramolecular hydrogen bond is formed between the NH group at the *ortho*-position of 2-phenyl group and the lone pair electrons of nitrogen on pyrimidine ring. Therefore, thirty one 4-anilinoquinazoline derivatives were synthesized. The interactions of synthesized compounds with *c-myc* G-quadruplex DNA were examined through fluorescence resonance energy transfer (FRET), circular dichroism (CD) spectroscopy, surface plasmon resonance (SPR), reverse transcription-polymerase chain reaction (RT-PCR), and Western blot. In addition, effects of these compounds on cell proliferation and cell cycle were also evaluated.

2. Chemistry

The synthetic routes for compounds **1a**–**b**, **2a**–**b**, **3a**–**c** and **4a**–**b** were shown in Scheme 1. The chlorination of 1*H*, 3*H*-quinazoline-2,4-dione with excess phosphorus oxychloride was carried out to give dichlorinated intermediate **10** [24]. Aniline substitution occurred selectively at C-4 position of **10**, yielding 4-aniline-2-chloroquinazoline **11** [25]. Reaction of **11** with acylchloride gave intermediates **12a** and **12b** [21], followed by treatment of **12a** or **12b** with excess diethylamine, potassium iodide, and potassium carbonate to give target compounds **1a** and **1b**, respectively. In comparison, **12a** or **12b** was treated with diethylamine and potassium carbonate to give target compound **2a** or **2b** as major product. Compounds **3a**–**c** were prepared via Suzuki coupling reaction of **2a** or **2b** with appropriate phenylboronic acid [26]. Intermediate **10** was reacted with 4-methoxyaniline to form intermediate **13**, followed by demethylation reaction with BBr_3 to give intermediate **14**, and subsequently by substituting with dibromoalkane to give intermediate **15a** or **15b**. Compounds **4a** and **4b** were obtained through reaction of **15a** or **15b** with diethylamine, respectively.

The synthetic routes for compounds **5a**–**f**, **6a**–**d**, **7a**–**i**, **8a**, **8b**, and **9a** were shown in Scheme 2. The synthetic method started with the synthesis of acylchloride through the chlorination of 2-methoxybenzoic acid or 2-nitrobenzoic acid, followed with its amidation using anthranilamide to give amide intermediate **16** or **22**, and subsequently, intermediate **17** or **23** was respectively prepared through the oxidative ring closure of **16** or **22** under basic conditions [21]. The chlorination of **17** with excess thionyl chloride was carried out to give intermediate **18** [24]. The chlorination of **23** with excess phosphorus oxychloride and phosphorus pentachloride was carried out to give intermediate **24**. Coupling of 4-methoxyaniline with **18** afforded intermediate **19**, followed with demethylation to give intermediate **20**. Compound **20** was reacted with 1,2-dibromoethane to afford intermediate **21** [27]. Coupling of