Tetrahedron 68 (2012) 5453–5457

Contents lists available at SciVerse ScienceDirect

Tetrahedron

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

Synthesis of bisquinoline–pyrrole oligoamide as G-quadruplex binding ligand

Chi Wi Ong [a,*], Meng-Chi Liu [a], Kun-Da Lee [a], Keng Wei Chang [a], Ya-Ting Yang [a], Hung-Wei Tung [a], Keith R. Fox [b]

[a] National Sun Yat-Sen University, No. 70, Lienhai Rd., Kaohsi0ung 804, Taiwan
[b] Centre for Biological Sciences, Life Sciences Building, University of Southampton, Southampton SO17 1BJ, United Kingdom

ARTICLE INFO

Article history:
Received 17 January 2012
Received in revised form 10 April 2012
Accepted 24 April 2012
Available online 9 May 2012

Keywords:
Bisquinoline
Polyamide
Quadruplex

ABSTRACT

A one-pot procedure using ammonium formate under palladium catalysis for the reductive dechlorination and reduction of nitro group of 4-chloro-8-nitro–quinoline derivatives has be successfully carried out. This has lead to the synthesis of bisquinoline–pyrrole oligoamide **1**, which show significant G-quadruplex selectivity in preference to duplex DNA. The cooperativity between the bisquinoline and pyrrole oligoamide moieties for good binding affinity to G-quadruplex was proven by synthesizing **2** and **3** lacking a quinoline ring and pyrrole amide, respectively, and both show much reduce affinity to G-quadruplex. Altogether, the results demostrate that the appropriate combination of two chromophores to form the hybride can attenuate binding affinity and selectivity towards G-quadruplex, an important criteria for the rational drug design.

1. Introduction

G-quadruplexes secondary structure are found in G-rich regions of DNA through the association of four guanine to form the guanine tetrad, followed by stacking of the guanine tetrad on top of each other.[1] The ability of G-rich sequences in telomeres and numerous gene promoters to form quadruplex structures has been exploited for the development of anticancer agents that promote or stabilize G-quadruplex.[2–6,14,23] The main strategy is the design of large flat aromatic ligands with or without containing cationic side chains that binds to the G-tetrad through π-π stacking and electrostatic interaction,[1b,3e,f,7] such as macrocyclic telomestatin,[8] pentacyclic acridinium ligands,[9] cationic porphyrin,[10] 4,7-diamino-1,10-phenanthroline derivatives[11] and bisquinolinium compounds.[12] However the grooves of quadruplex structures remains an opportunity for ligands binding. More recently, distamycin, a DNA minor groove binding agent has been found to interact with the grooves of quadruplexes.[13] Till today, the design of quadruplex binding ligands has mainly focused individually on π–π stacking at the G-tetrads or hydrogen bond at the groove, while their cooperative interaction has rarely been discussed.[14]

Quinoline oligoamides has been reported to fold into predictable and very stable secondary motifs,[15a,b] and recently shown to be a good candidate as G-quadruplex ligands.[15c] The tetramer of 8-amino-2-quinoline carboxylic acid with a 1.5 turn helically folded architecture has been shown to have significant binding affinity and selectivity for G-quadruplex without any evidence of DNA-duplex binding.[15c] It should be noted that the 4-position of the quinoline ring contain a cationic side chains to confer electrostatic interaction with the G-quadruplex, and possibly improved water solubility. Interestingly, the bisquinoline adopting a planar crescent-like structure was found to be a poor G-quadruplex ligand. Can the π-rich bisquinoline be transform into a good G-quadruplex ligand? Herein, we hope to use cooperative effect by incooperating pyrrole oligoamide to the bisquinoline and study the ability to stabilize G-quadruplex.

In this contribution we demonstrated that the incorperation of pyrrole oligoamide to bisquinoline in **1** favours G-quadruplex stabilization. As shown in Fig. 1, the bisquinoline can adopt a near planar conformation (**1b**), whereby playing the role of π–π stacking with the G-tetrads, and the pyrrole oligoamide further attenuating the G-quadruplex stabilization. We next need to offer prove of cooperativity between the bisquinoline and pyrrole amide moieties for the good binding affinity and selectivity towards G-quadruplex. The role of π–π stacking ligand for bringing the groove binding ligand to close proximity was further tested by replacing one quinoline ring of compound **1** with a phenyl ring in compound **2**. By replacing one of the quinoline ring of compound **1** with a phenyl ring to form compound **2** we have reduced the available π–π stacking interaction. Compound **3** was constructed to contain two quinolines rings tethered to dimethylaminopropane, but without an attached pyrrole amide. Both compound **2** and **3** have a lower binding affinity fo G-quadruplex. Furthermore, our compound **1**–**3** do not possess a functional group at 4-position of

* Corresponding author. Tel.: +886 952311150; fax: +886 7 5253908; e-mail address: cong@mail.nsysu.edu.tw (C.W. Ong).

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2012.05.001

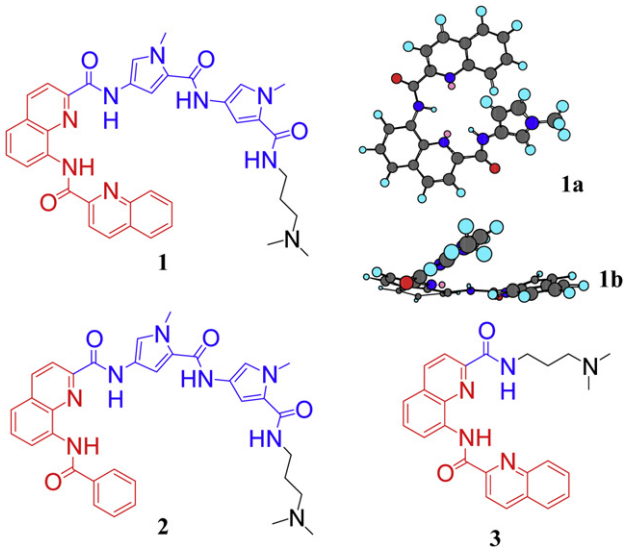


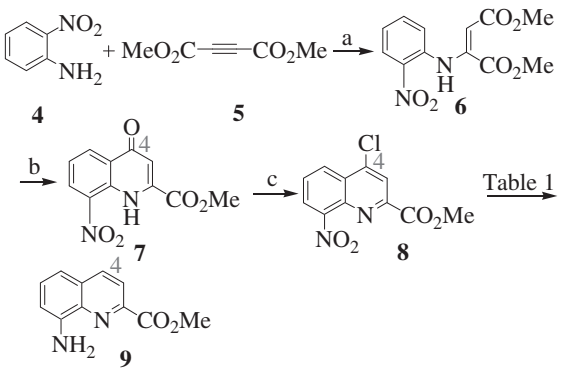
Fig. 1. Compounds **1**–**3** to probe the cooperative between guanine tetrad π–π stacking ligand and DNA minor goove binding ligands. **1a** and **1b**, top and side view of **1** with one pyrrole ring showing the near planarity of the bisquinoline rings with the pyrrole ring sticking outwards.

quinoline that may aid G-quadruplex stabilization. We have found an expedient method for silencing the 4-position of quinoline by converting 4-chloro-8-nitro-quinoline derivative to the 8-amino-quinoline derivatives under hydrogenation condition, a one-pot procedure for the reductive dechlorination with consecutive reduction of nitro group to amine.

2. Results and discussion

Our synthesis began with the reaction of 2-nitroaniline **4** with dimethyl-acetylenedicarboxylate **5** give **6**, followed by cyclization to **7** and aromatization to give 4-choro-8-nitro-quinoline-2-carboxylic ester **8** using reported procedures,[16] but with slight modifications (Scheme 1). We found that the cyclization of compound **6**–**7** was best performed using a 1:2 w/w ratio of compound to polyphosphoric acid at 180 °C for a short reaction time (<1 h), whereby prolong reaction time gave a much lower yield. Our first task was to dehalogenate compound **8** using several reported reductive delogenation conditions as shown in Table 1. The use of sodium amalgam or copper powder in organic acid[17] failed to give the required product. Interestingly, the use of ammonium formate with Pd/C catalysis[18] for the dechlorination of **8** was found to simultaneously reduced the nitro group to the amine in one-pot to give directly the key intermediate **9**. Compound **9** was subsequently reacted with benzoyl chloride and quinoline-2-carbonyl chloride in the presence of DIPEA to give **10** and **11**, respectively, in high yield. The ester of **10** and **11** was hydrolyzed to the acid and then converted to the acid chloride **10a** and **11a**, respectively, for the subsequent preparation of our model **1**–**3**. The coupling of **10a** and **11a** with the previous reported pyrrole oligoamide **12**[19] gave the desired product **1** and **2**, respectively, in moderate yield. Similarly reaction of **11a** with *N*,*N*-dimethylaminopropylamine gave **3** (Scheme 2).

We next investigated the ability of these compounds **1**–**3** to bind to duplex DNA and G-quadruplex. The comparative thermal stabilization (ΔT_m) of calf thymus (CT) duplex by compounds **1**–**3** was performed using UV absorbance–temperature plot under identical conditions of buffer and pH. The results show that **3** displayed slight binding for CT DNA (ΔT_m=0.7 °C) as compared with **1** and **2** (ΔT_m<0.3 °C) (see ESI). Deoxyribosenuclease I footprinting analysis is a useful technique for locating the specific binding site of small molecule on DNA. Footprinting of compounds **1**–**3** with Hex B



Scheme 1. Preparation of quinoline motif. (a) MeOH, rt, 18 h then reflux 6 h, 82%. (b) PPA/**6**=2.26, 180 °C, 45 min, 84%. (c) $POCl_3$, reflux 2 h then rt, 1 h, 95%.

Table 1
Dehalogenation of **8**

Entry	Conditions	Product
1	Na(Hg), rt, 4 h in THF/MeOH=1:1	*
2	Hexanoic acid, Cu power, 160–170 °C, 10 min.	**7**
3	Ammonium formate, 5% Pd/C, in MeOH, reflux 10.5 h	**9**

● Complex mixtures.

show no protection mapping with concentration greater than 10 μM. This substatiate the comparative thermal stabilization results, which showed that compounds **1**–**3** have minimal affinity to duplex DNA.

The poor binding to duplex DNA of **1**–**3** upto a concentration of over 10 μM encourages us to investigate whether these compounds can behave as a good selective G-quadruplex binding agent. The stabilization to various G-quadruplexes was also investigated by fluorescence melting analysis[20] using human telomeric G-quadruplex (HT), *c-kit* and *c-myc* promoters. The thermal melting temperatures of the various quadruplexes were determined using the fluorescence melting technique developed previously by Darby et al.[21a] for assessing the stability of related quadruplexes[21b–e] in the present or absent of ligands. The stabilization of the quadruplex increases its melting temperature (ΔT_m). At the concentration of 2 μM, distamycin and compound **12** showed no stabilization for all the G-quadruplex in this study (see ESI). As such, a concentration of 2 μM for ligand **1**–**3** was chosen to study their ability to stabilize G-quadruplex. The synthesized ligand **1**–**3** showed good to moderate stabilization of the G-quadruplex and the results are shown in Fig. 2 and Table 2. The T_m enhancement (ΔT_m) for each compound is tabulated in Table 2.

Our results indicate clearly that ligand **1** was the best in stabilizing all the G-quadruplex, with a ΔT_m value of 7 °C for HT, 5.4 °C for *c-kit*, and 4.1 °C for *c-myc*, respectively. Compound **2**, with reduced π–π stacking, while compound **3**, which lacks the bispyrrole oligoamide, in our design strategy were uniformly weaker G-quadruplex binders. To our delight, the bisquinoline pyrrole oligoamide hybrid **1** has a cooperative effect in enhancing binding affinity and selectivity for G-quadruplex.

Recently, there have been growing interest to design small molecules that show some discrimination between G-quadruplexes.[22–25] These results provide some hints for designing ligand to discriminate between G-quadruplexes ligands based on π-rich bisquinoline and pyrrole oligoamide. For the HT sequence, the ΔTm produced by **1** is greater than that for **2** and **3**. This suggests that both π–π stacking and pyrrole oligoamide are required for optimal binding to HT sequence. Compound **1** and **3** has a higher ΔTm than **2** with the *c-kit* sequence, this indicate that π–π stacking is playing a more predominant role for binding to *c-kit* sequence.