Synthesis of potent G-quadruplex binders of macrocyclic heptaoxazole and evaluation of their activities

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ABSTRACT

Guanine-rich DNA sequences form unique three-dimensional conformation known as G-quadruplexes (G-q). G-q structures have been found in telomere and in some oncogene promoter. Recently, it was suggested that G-q showed some biological activities including telomere shortening and transcriptional regulation. In this paper, we synthesized selective G-q binders and evaluated of their biological activities.

INTRODUCTION

Human telomeres are located at the ends of chromosomes containing repeating (TTAGGG)ₙ sequences, whose 3’-ends exists as a single-stranded overhang. This single G-Rich strand forms a characteristic four-stranded helical conformation, called G-quadruplexes (G-q), in the presence of high concentrations of monovalent cations, such as potassium or sodium ions.¹ Telomeric G-q formation shortens the telomere drastically following the dissociation of TRF2 and/or Pot1, which bind at the end of the telomere, thereby inducing apoptosis of cancer cells. Since potent and sequence-selective G-q binders would be candidate anti-cancer agents, as well as useful biological tools, a number of studies have been conducted aimed at the development of potent G-q binders. Telomestatin (TMS) (1), which has a macrocyclic structure containing five oxazoles, two methyl oxazoles, and a thiazoline, is a natural product isolated from Streptomyces amatus 3533-SV4.² The macrocyclic poly-oxazole structure of TMS (1) was reported to interact with telomere, strongly stabilizing the G-q structure. We have recently synthesized 60TDs 2-5 (Scheme 1), having a C₃-symmetrical macrocyclic hexaoxazole structure with various functional groups, as analogs of 1.³,⁴ (structure-activity relationships) SAR studies on 60TDs indicated that the functional groups play a critical role in the stabilization of G-q structure. Here, with focusing on improving the planarity of 60TDs, we newly designed L1H1-70TD (6), which has a macrocyclic heptaoxazole with an amino group on its side chain. We anticipated that the more planar macrocyclic structure compared to 60TDs would allow strong intercalation to G-q through π-π interactions. The amino group in 6 was also expected to stabilize the G-q structure efficiently through interaction with phosphate in G-q. In this paper, we describe the synthesis of L1H1-70TD (6) and evaluation of its biological activities.

RESULTS AND DISCUSSION

L1H1-70TD (6) was synthesized as shown in scheme 2. The trioxazoles 7 and 8 were prepared as previously reported and the corresponding macrocyclic bisamide 9 was synthesized by 6 steps.²,³ The TBS group of 9 was deprotected with HF-pyridine to give the alcohol 10. Construction of the seventh oxazole ring from 10 into 13 was troublesome because of the strained structure of the β-hydroxyamidine moiety. After many attempts, it was achieved by modifying the Pattenden protocol. Thus, alcohol 10 was converted into enamide 11 by mesylation, followed by treatment with DBU. The resulting enamide 11 was converted to heptaoxazole 13 through oxazoline 12 by reaction with NBS and Cs₂CO₃ in acetonitrile. Finally, the Boc group was deprotected with TFA to give L1H1-70TD (6) in 10% yield from 11 (Scheme 2).³ With the macrocyclic heptaoxazole derivative L1H1-70TD (6) in hand, we examined the interaction of 6 with telomeric DNA by CD spectra analysis using ss-telo24. Upon treatment of 6 with ss-telo24, the CD spectrum changed, and a positive peak at 292 nm and a negative peak at 262 nm were observed. These characteristic spectral changes clearly showed that L1H1-70TD (6) induced a change of
the ss-telo24 conformation to anti-parallel G-q (Fig. 1, open circle). Interestingly, L1H1-7OTD (6) was found to convert the parallel/anti-parallel mixed type structure of ss-telo24, induced by potassium cation (Fig. 1 black square), into the anti-parallel G-q structure (Fig. 1 open square). Therefore, L1H1-7OTD (6) was revealed to strongly induce the ss-telo24 to an anti-parallel G-q structure. To verify the above anti-parallel G-q structural change was “intramolecular” mode, (electrophoresis mobility shift assay) EMSA was carried out using ss-telo24 with 6. At the higher concentrations, the new band with high mobility which corresponds to the “intramolecular” G-q structure was significantly increased with 6 and (Fig. 2). EC_{50} values of L1H1-7OTD (6) was evaluated, and was found to be 151 ± 16 μM under the ss-telo24 concentration of 50 μM. Next, selective interaction of L1H1-7OTD (6) with the telomeric DNA sequence was examined by using (polymerase chain reaction) PCR stop assay with ss-telo24 and its mutant sequence ss-telo24 mut. Under the protocol, the selectivity of 6 was evaluated by PCR inhibitory activity. In this experiment, L1H1-7OTD (6) showed strong inhibition of the extension of ss-telo24, with an IC_{50} value of 0.67 ± 0.01 μM, and weak inhibition of ss-telo24 mut with 5.2 ± 0.8 μM, indicating that L1H1-7OTD (6) selectively interacts with ss-telo24.  

CONCLUSION

In conclusion, we have developed macrocyclic heptaoxazoles, L1H1-7OTD as G-q binders. This planer G-q binder strongly and selectively interacted with ss-telo24 and induced conformational change to intramolecular anti-parallel G-q structure. The macrocyclic

Fig. 1 CD spectra of 10 μM ss-telo24 in Tris-HCl buffer (50 mM pH 7.6) with 50 μM 7OTDs and/or 100 mM K^+. Black triangle = ss-telo24 (no salt added); open circle = ss-telo24 + L1H1-7OTD (6) (no salt added); black square = ss-telo24 + KCl; open square = ss-telo24 + KCl + L1H1-7OTD (6).

Fig. 2 Effect of L1H1-7OTD (6) on the formation of intramolecular G-q. ss-telo24 (50 μM) was incubated for 60 min with various concentrations of L1H1-60TD (6) in 50 mM Tris-HCl buffer (no salt added). After incubation, samples were mixed with ficoll 400 and run a 12% native PAGE with 1 x TBE at 4 °C. All oligonucleotides were stained by Stains-all.

heptaoxazole structure appears to be a powerful scaffold for stabilizing the telomere anti-parallel G-q.

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REFERENCES


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