SYNTHESIS OF MACROCYCLIC PENTA- AND TETRAOXAZOLES AS G-QUADRUPLEX LIGANDS†

Shadi Sedghi Masoud, Yamato Tsushima, Keisuke Iida, and Kazuo Nagasawa*

Department of Biotechnology and Life Science Faculty of Technology, Tokyo University of Agriculture and Technology (TUAT), Koganei, Tokyo 184-8588, Japan

Abstract – The penta- and tetraoxazole telomestatin analogs L2H2-5OTD (4) and L2H2-4OTD (5) were synthesized as new G-quadruplex ligands in order to evaluate the influence of the size of the planar macrocycle on the G-quadruplex-stabilizing efficacy. These ligands were less potent stabilizers of various G-quadruplex-forming oligonucleotides than L2H2-6OTD (2a), which has a hexaoxazole-type macrocycle.

Guanine-rich DNA sequences in telomeric DNA and the promoter regions of some cancer-related genes, such as c-kit, bcl-2, c-myc, and k-ras, form characteristic higher-order G-quadruplex structures, which play significant roles in regulation of enzyme functions and/or gene transcription. For example, telomerase activity is inhibited by formation of G-quadruplex structures in telomeric DNA sequences, and dissociation of telomere-related proteins such as TRF2 and Pot1 from 3’-overhang chromosomes induces apoptosis of various cancer cells. Stabilization of G-quadruplex structure is currently considered a promising approach for cancer treatment, and a number of small compounds with G-quadruplex-stabilizing activity (G-quadruplex ligands) have been developed. We have recently synthesized macrocyclic hexaoxazoles (6OTDs, 2) as G-quadruplex ligands inspired by the natural G-quadruplex ligand telomestatin (TMS) (1). Telomestatin (1) shows potent telomerase-inhibitory activity by stabilizing telomeric G-quadruplex structure through π-π interaction with the planar G-quartet in an end-stacking mode. Since 6OTD (2) has similar size and planarity to 1, they are expected to interact with G-quadruplex structures in a similar manner to 1. Recently, we analyzed the interaction
mode of telomeric G-quadruplex with L2H2-6OTD (3), which has potent G-quadruplex-stabilizing ability similar to that of TMS, by means of an NMR study, and confirmed that it interacts with the G-quartet in an end-stacking mode through π-π interaction.\textsuperscript{11} Other macrocyclic G-quadruplex ligands are also considered to interact similarly with the G-quartet.\textsuperscript{12} Although the size of the macrocycle seems likely to be important for the stabilization efficacy, little is known about the structure-activity relationship from the viewpoint of macrocycle size.\textsuperscript{12b,12c} In this paper, we describe the synthesis of L2H2-5OTD (4) and L2H2-4OTD (5), which bear smaller penta- and tetraoxazole macrocycles, respectively, compared to the L2H2-6OTD (3) (Figure 1). The G-quadruplex-stabilizing ability of these ligands was evaluated by means of fluorescence resonance energy transfer (FRET) melting assays.

![Figure 1. Structures of telomestatin (1) and macrocyclic polyoxazole analogs (2-5)](image-url)
Synthesis of L2H2-5OTD (4) and L2H2-4OTD (5) was commenced with preparation of bisoxazoles 9a and 9b (Scheme 1). A carboxylic acid-bearing mono-oxazole 6 was reacted with serine methylester (7) in the presence of 4-((4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) and N-methylmorpholine (NMM) to give β-hydroxy amide 8 in 92% yield. Cyclodehydration of 8 with diethylaminosulfur trifluoride (DAST) followed by oxazole formation with bromotrichloromethane in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave bisoxazole 9 in 78% yield from 8. Then, carboxylic acid 9a and amine 9b were synthesized from 9 by hydrolysis of the methyl ester with lithium hydroxide or deprotection of Cbz group under hydrogen in the presence of Pd/C, respectively.

Scheme 1. Synthesis of bisoxazoles 9a and 9b

Synthesis of L2H2-5OTD (4) is depicted in Scheme 2a. Bisoxazole amine 9a was reacted with trioxazole carboxylic acid 10 in the presence of NMM and DMT-MM to give amide 11 in 94% yield. Deprotection of the Cbz group in 11 followed by hydrolysis of methyl ester gave an amino acid, which was subjected to macrocyclization using 2-methyl-6-nitrobenzoic anhydride (MNBA) in the presence of triethylamine (TEA) and 4-dimethylaminopyridine (DMAP) under high dilution conditions (3 mM) in CH2Cl2-DMF (2:1) to give 13 in 54% yield from 11. The Boc group was deprotected with TFA to give L2H2-5OTD (4) in 98% yield. Macrocyclic tetraoxazole L2H2-4OTD (5) was similarly synthesized from 9a and 9b via 14, 15, and 16 (Scheme 2b).
Scheme 2. Synthesis of a) L2H2-5OTD (4) and b) L2H2-4OTD (5)

The G-quadruplex-stabilizing ability of L2H2-5OTD (4) and L2H2-4OTD (5) was examined and compared with that of L2H2-6OTD (3) by means of FRET melting assay. In this assay, ligand-induced stabilization of a folded G-quadruplex is evaluated in terms of the increment of the
melting temperature, $\Delta T_m$. The $\Delta T_m$ values of the G-quadruplex-forming DNA sequences of telo21, \textit{bcl-2}, \textit{c-kit}, \textit{c-myc}, and \textit{k-ras} (0.2 $\mu$M) were measured in the presence of \textit{3}, \textit{4} and \textit{5} (2 $\mu$M), and the results are summarized in Table 1. In the case of telo21, the $\Delta T_m$ values were found to be 20.7, 14.9 and 5.1 °C in the presence of \textit{3}, \textit{4} and \textit{5}, respectively. The G-quadruplex-forming sequences of \textit{bcl-2}, \textit{c-kit}, \textit{c-myc}, and \textit{k-ras} showed similar trends. Thus, the size of the macrocyclic structure is clearly significant for polyoxazole G-quadruplex ligands, and the stabilizing ability of these ligands decreases as the size of the structure is reduced.

Table 1. $\Delta T_m$ (°C) values in FRET melting assay$^{a-c)}$

<table>
<thead>
<tr>
<th>Ligand</th>
<th>\textit{telo21}$^{d)}$</th>
<th>\textit{bcl-2}$^{d)}$</th>
<th>\textit{c-kit}$^{d)}$</th>
<th>\textit{c-myc}$^{d)}$</th>
<th>\textit{k-ras}$^{d)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{3}</td>
<td>20.7</td>
<td>18.1</td>
<td>22.1</td>
<td>21.7</td>
<td>16.1</td>
</tr>
<tr>
<td>\textit{4}</td>
<td>14.9</td>
<td>11.5</td>
<td>15.8</td>
<td>11.0</td>
<td>8.5</td>
</tr>
<tr>
<td>\textit{5}</td>
<td>5.1</td>
<td>2.4</td>
<td>7.6</td>
<td>2.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

$^{a)}$The FRET melting assays were conducted with DNA (0.2 $\mu$M) and ligands (2.0 $\mu$M) in the presence of K$^+$ (60 mM). $^{b)}$$\Delta T_m$ values were obtained in two independent experiments each performed in duplicate. $^{c)}$The G4-forming DNA sequences used in FRET melting assay are shown in Table 2. $^{d)}$$T_m$ values of \textit{telo21}, \textit{bcl-2}, \textit{c-kit}, \textit{c-myc}, and \textit{k-ras} in the absence of ligands were found to be 62.4 °C, 69.7 °C, 63.6 °C, 77.4 °C, and 64.9 °C, respectively.

Table 2. G4-forming DNA sequences used in FRET melting assay

<table>
<thead>
<tr>
<th>Oligomer name</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{telo21}</td>
<td>GGGTTAGGGTTAGGGTTAGGG</td>
</tr>
<tr>
<td>\textit{bcl-2}</td>
<td>GGGCGCGGGAGGAAGGGCGGG</td>
</tr>
<tr>
<td>\textit{c-kit}</td>
<td>GGGAGGGCGCTGGGAGGAGGG</td>
</tr>
<tr>
<td>\textit{c-myc}</td>
<td>GAGGGTGAGGAGGAGGGAGG</td>
</tr>
<tr>
<td>\textit{k-ras}</td>
<td>AGGGCGGTTGGAAGGGAGGAGGG</td>
</tr>
</tbody>
</table>

In conclusion, we have synthesized novel G-quadruplex ligands L2H2-5OTD (4) and L2H2-4OTD (5) bearing pentaoxazole and tetraoxazole core structures, respectively. Examination of the effect of the size of macrocyclic structure on G-quadruplex-stabilizing activity revealed that hexaoxazole structure is optima among these three sizes, and the stabilizing ability decreases with decreasing size of the macrocycle.
ACKNOWLEDGEMENTS

This work was supported in part by Grants-in-Aid for Scientific Research (B) from JSPS (23310158 and 26282214) and a Grant-in Aid for Challenging Exploratory Research from JSPS (21655060). K.N. is grateful for financial support from the Mukai Science and Technology Foundation, Tokyo (Japan), and the Mochida Memorial Foundation for Medical and Pharmaceutical Research, Tokyo (Japan). K.I. is grateful for financial support in the form of JSPS Predoctoral Fellowships for Young Scientists.

REFERENCES AND NOTES

†We would like to dedicate this paper to Professor Dr. Isao Kuwajima on the occasion of his 77th birthday.


6 (a) T. A. Brooks, S. Kendrick, and L. H. Hurley, FEBS J., 2010, 277, 3459; (b) D. Z. Yang and K.


17 Spectral data for L2H2-5OTD (4): $[\alpha]_b^{25}$ -6.5 (c 1.9, MeOH); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.12 (s, 1H), 9.07 (s, 1H), 9.06 (s, 1H), 8.90 (s, 1H), 8.89 (s, 1H), 8.81 (d, $J = 6.0$ Hz, 1H), 8.65 (d, $J = 6.0$ Hz, 1H), 7.75 (brs, 4H), 5.35 (m, 2H), 2.74 (m, 4H), 2.16 (m, 2H), 1.96 (m, 2H), 1.57 (m, 4H), 1.40 (m, 2H), 1.13 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 164.3, 164.2, 158.9, 158.7, 155.2, 154.3, 154.2, 142.5, 142.3, 141.8, 140.7, 139.9, 136.4, 135.7, 129.3, 128.5, 128.0, 48.1, 48.0, 38.6 (2 carbons), 32.6, 32.2, 26.7 (2 carbons), 20.8, 20.6 ppm; HRMS (ESI, M+H) cacld for C$_{27}$H$_{30}$N$_9$O$_7$ 592.2268, found 592.2234.

18 Spectral data for L2H2-4OTD (5): $[\alpha]_b^{25}$ -10.2 (c 1.5, MeOH); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.02 (s, 1H), 9.00 (s, 1H), 8.85 (s, 2H), 8.76 (s, 2H), 7.84 (brs, 4H), 5.29 (m, 2H), 2.80 (m, 4H), 2.06 (m, 4H), 1.60 (m, 4H), 1.43 (m, 4H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 165.4, 159.9, 154.7, 142.8, 141.1, 136.5, 128.8, 46.2, 38.6, 30.9, 26.5, 22.3 ppm; HRMS (ESI, M+H) cacld for C$_{24}$H$_{29}$N$_8$O$_6$ 525.2210, found 525.2236.