Cationic corrole derivatives: a new family of G-quadruplex inducing and stabilizing ligands†

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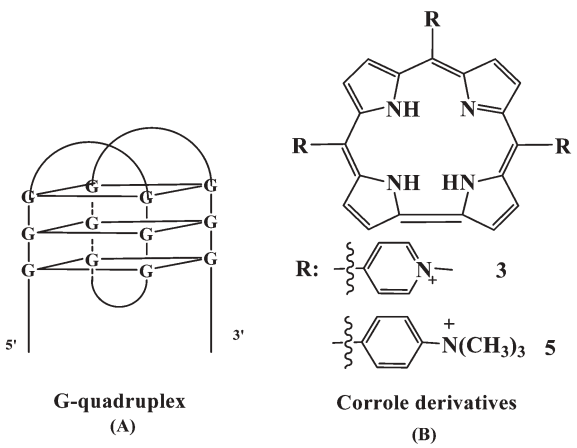
Water-soluble cationic corrole derivatives were designed and synthesized, and the first observation of their interactions with the telomeric G-quadruplex was made.

Telomeres exist at the ends of eukaryotic chromosomes and can protect the chromosomes, which play very important roles in many aspects of cells.[1,2] It is known that telomeric repeats with the single-stranded G-rich overhang can be extended by telomerase.[3] As telomerase is overexpressed in most tumor cells and not in normal cells, such a unique enzyme could be a good target for the design of antitumor drugs with high selectivity.[3] Meanwhile, a single-stranded 3′ overhang of telomeric DNA[4] can be folded into intramolecular G-quadruplex structures leading to hindrance of the reaction of telomerase.[5] Therefore, if a ligand could stabilize the G-quadruplex (Scheme 1A), it could have a potential application as a telomerase inhibitor. Recently, many G-quadruplex stabilizers have been synthesized and studied for their biological and medicinal activities by many groups.[6–9]



Scheme 1 Structures of (A) G-quadruplex and (B) pyridinium corroles.

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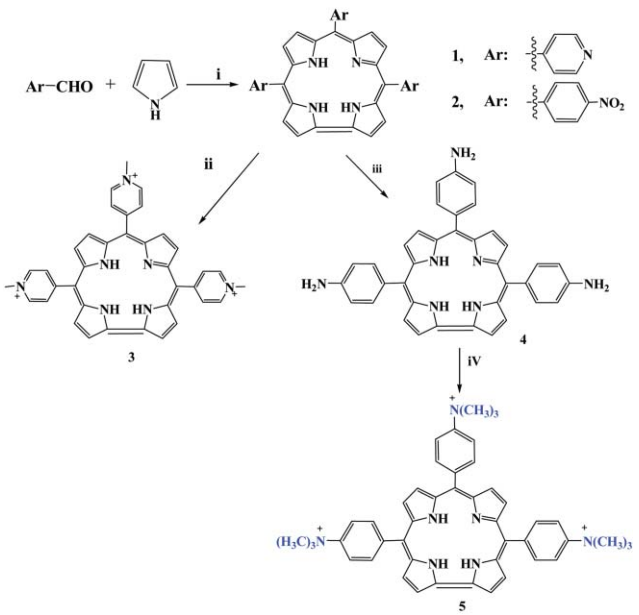
Corroles are tetrapyrrole macrocycles with one of the *meso*-carbon atoms substituted by a direct pyrrole–pyrrole link.[10] It has been found that corroles can be applied in synthesis, catalysis, biological and medicinal studies.[11] However, few reports of corroles in medicinal or biological applications have been published.[11] In this paper, we shall report our synthesis of cationic corrole derivatives **3** and **5** (Scheme 1B) and our first findings of their good abilities to stabilize the G-quadruplex structure as tested by polymerase stop assay, SPR experiments and CD melting. Furthermore, their capabilities to induce G-quadruplex structure formation were tested by CD.

Gross's group reported a kind of 2-pyridinium pentafluorophenyl corrole.[11b] In our synthesis of 4-pyridinium corrole **3**, we obtained this compound in two steps by a modification of the reported method.[12] Pyrrole condensed with 4-pyridyl-carboxaldehyde to form 4-pyridyl corrole **1** in acetic acid, then 4-pyridyl corrole was methylated in DMF to form water-soluble 4-pyridinium corrole **3** in high yield (Scheme 2).

Synthesis of corrole **5** was accomplished by condensing pyrrole with 4-nitrobenzaldehyde to form 4-nitrophenyl corrole in acetic acid.[12] Then 4-nitrophenyl corrole was reduced by $SnCl_2$ and



Scheme 2 Synthesis of pyridinium corroles. *Reagents and conditions*: i) acetic acid, 110 °C for 4 h, 2.6% yield for **1**, 13.5% for **2**; ii) MeI, DMF, rt, overnight, 87% yield; iii) $SnCl_2{\cdot}2H_2O$, 37% aq. HCl, 38% yield; iv) MeI, acetone, 60 °C, 7 days, 65% yield.

4-amino corrole **4** was obtained.[11b] Methylation of corrole **4** was achieved in acetone at 60 °C for seven days and quaternary ammonium corrole **5** was obtained in good yield.

All the new compounds were fully characterized by NMR, UV, elementary analysis and HRMS (see Supporting Information). As in methylation of pyridyl corrole **1** in DMF, mono-anionic forms in the inner nitrogens of corrole **2** were observed by ESIMS. This is consistent with the early results reported by Gross.[13]

To identify if corroles **3** and **5** could stabilize the G-quadruplex structure, a *Taq* DNA polymerase stop assay was used to determine the corrole-induced stability of the quadruplex based on the template strand at low K^+ concentration.[14] The experimental results indicated that the pausing site in the DNA extension process was enhanced by stabilizing the G-quadruplex structure by corrole induction (Fig. 1). This suggested that corroles **3** and **5** could stabilize the G-quadruplex structure.[8a,9a]

Further experimental data from the CD melting method supported this conclusion.[9b] Our results indicated that both corroles **3** and **5** were able to induce strongly the stabilization of quadruplex DNA (sequence 5′-CATGG TGGTTT GGGTTA GGGTTA GGGTTA GGGTTA CCAC-′3; T_m = 43 °C in the absence of corrole). During our experiments, CD-thermal transitions were monitored at 295 nm. We found a reverse sigmoidal pattern and this is the property of intramolecular G-quadruplex formation (Fig. 2).[15] In the presence of corroles **3** and **5**, the melting temperature increased by 38 °C and 16 °C respectively. Data suggested that corroles **3** and **5** strongly stabilized the G-quadruplex in Na^+ buffer.

The binding abilities of corroles **3** and **5** to human telomeric sequence, 5′-$AG_3TTAG_3TTAG_3TTAG_3$ and three other immobilized duplex DNA (GC, AT, ds) were examined by SPR (surface plasmon resonance).[7] The steady-state equilibrium binding

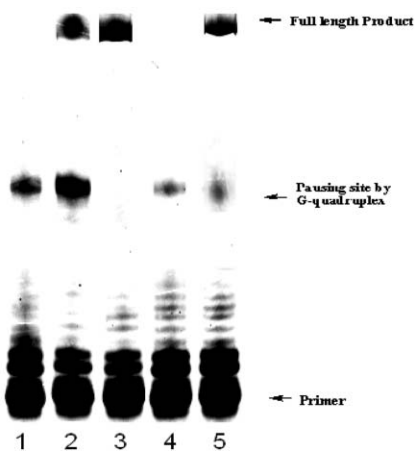


Fig. 1 Concentration-dependent block of *Taq* polymerase DNA synthesis by the G-quadruplex structure formed on the HT4 template at 55 °C. Primer (P18) labeled with Tamra was mixed with template DNA (HT4) in a Tris-HCl buffer (10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$) containing 50 mM K^+. Lane 3, control. Lanes 1, 2, concentration effect of corrole **3**, 2.5, 1 μM. Lane 4, corrole **5** 2.5 μM. Lane 5, **TMPyP4** 2.5 μM. The sequencing gel showed the enhanced DNA synthesis pausing at the G-quadruplex site. Arrows indicate the positions of the full-length product of DNA synthesis, the G-quadruplex pausing site and the free primer. The products were separated on a 20% denaturing polyacrylamide gel for electrophoresis.

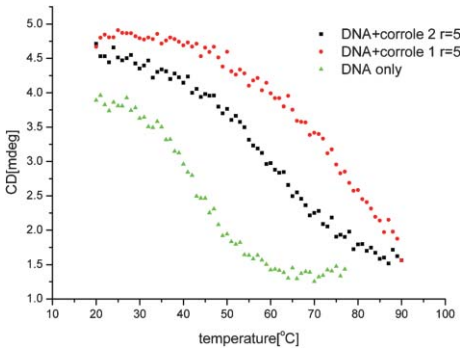


Fig. 2 CD Melting curve of 10 μM DNA oligonucleotide in 10 mM Tris-HCl at pH 7.4, 100 mM NaCl, 1 mM EDTA buffer. CD spectra were monitored at 295 nm. Curve color: green = DNA only. Black = corrole **5** + DNA(r = 5). Red = corrole **3** + DNA(r = 5). r = corrole/DNA strand concentration.

constants and kinetic constants of corroles **3**, **5** with duplex and quadruplex DNA were both measured under previously described experimental conditions.[16] (Fig. 3 and Figs. S1, S2, see Supporting Information) The sensorgrams indicated that corroles **3** and **5** had high binding constants, and obvious selectivity for quadruplex DNA. (Table S1, see Supporting Information) From our experimental data, we found that the selectivities of corroles **3** and **5** were much better than that of TMPyP4[8d](5,10,15,20-tetrakis-(*N*-methyl-4-pyridyl)-21*H*,23*H*-porphyrin), which is a good G-quadruplex stabilizer.[8b]

It has been reported that DNA oligonucleotide with a human telomeric sequence could form an intramolecular basket-type G-quadruplex structure in the presence of Na^+, while in K^+ solution, the conformation might be a hybrid of parallel/anti-parallel G-quadruplex structures.[17] These conformation changes could be observed by CD measurement. A typical antiparallel G-quadruplex structure of $d[T_2AG_3]_4$ oligonucleotide was formed in the presence of Na^+ which exhibits a positive band near 295 nm and a negative band at 265 nm in CD spectra.[17,19] In the presence of K^+, both a small peak at 265 nm and a stronger positive band around 288 nm are the characteristics of the hybrid G-quadruplex

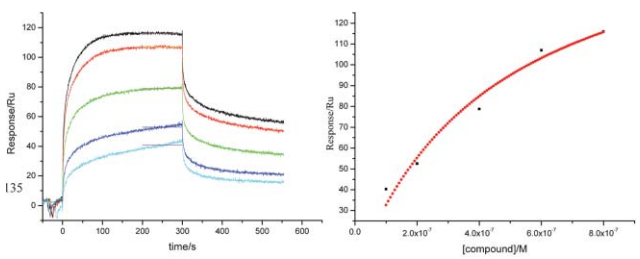


Fig. 3 (a) SPR sensorgram overlay for binding of corrole **3** to G-quadruplex at 25 °C. The unbound ligand concentrations in the flow solution were 100, 200, 400, 600, 800 nM from the lowest curve to the top curve. (b) General fit of 100 s steady-state region and the corresponding fitting curves. Binding curve with G-quadruplex obtained using the BIAeval V3.1 software (BIAcore). The SPR experiments were carried out in pH 7.4, 0.01 M Hepes, 0.2 M KCl, 3 mM EDTA, 0.005% (vol/vol) surfactant P20 running buffer using a streptavidin functionalized chip on a BIAcore X optical biosensor, as described previously.[9a]

in CD spectra.[18,19] On the other hand, for the control experiment, the intrinsic peak of $d[T_2AG_3]_4$ oligonucleotide at 257 nm without cation was observed in the CD spectra, which suggested that G-quadruplex structures could not be formed without an ion.[10]

In our current studies, we surprisingly found that corroles **3** and **5** could induce the formation of the G-quadruplex without an ion. The peaks at 257 nm of CD spectra were gradually shifted to 290 nm (Fig. S4 a, see Supporting Information) with the increase of concentration of corrole **3** in $d[T_2AG_3]_4$ oligonucleotide Tris-HCl solution. These peaks are consistent with CD spectra of the $d[T_2AG_3]_4$ oligonucleotide in K^+ buffer,[19] which suggested the formation of a hybrid parallel/antiparallel G-quadruplex in the buffer without ion. The same phenomenon was also observed for corrole **5** (Fig. S3, see Supporting Information).

Further studies on the G-quadruplex structural transition induced by corrole **3** in the presence of Na^+ and observed by CD were carried out. When corrole **3** was added, the signal absorbance at 265 nm increased greatly from a negative band to a small peak. However, there are almost no changes in the positive band at 295 nm. (Fig. S4 b, see Supporting Information) These results supported the proposal that the structure transition from antiparallel to mixed-type hybrid G-quadruplex was induced by corrole **3**.

To the best of our knowledge, only a few small molecules can influence the structural transition of a human telomeric sequence and induce the formation of a G-quadruplex structure.[9,17,20,21] The CD spectroscopy measurement demonstrated here that corrole **3** not only could induce human telomeric $d[T_2AG_3]_4$ oligonucleotide to form the mixed-type hybrid G-quadruplex structures, but also transfer the antiparallel structure to a hybrid parallel/antiparallel G-quadruplex structure.

The compounds were further tested for their inhibiting effect on telomerase activities by the TRAP method. The TRAP assays were performed according to the reported method.[22] The IC_{50} values of corroles **3** and **5** are 8.6 μM and 4.4 μM respectively.

Qualitative molecular modeling using molecular mechanics energy minimizations was studied and experimental results showed good overlaps between corrole **3** or **5** and guanine quartets (Fig. S4 and Fig. S5, see Supporting Information). It predicted that the aromatic rings with positive charges were tilted from the plane of the ligand to follow the twist of the grooves and the groups with positive charges were directed into the grooves toward the sugar–phosphate backbone.

In conclusion, the water-soluble cationic corroles **3**, **5** were designed and synthesized, and the first observations of their interactions with the telomeric G-quadruplex were made. Experimental results indicated that these compounds could be a new class of G-quadruplex stabilizers. SPR sensorgrams showed the obvious selectivities of corroles **3**, **5** for G-quadruplex DNA compared to ds DNA. The formation and transition of the G-quadruplex induced by corroles **3** and **5** were observed by CD. The high affinities of corroles **3**, **5** for quadruplex DNA are associated with the planar arrangement of the rings, their appropriate spacing and the substituent groups with positive charges based on the molecular modeling results. These studies suggested that the water-soluble cationic corrole derivatives might be potential candidates for new anticancer agents. Further investigation of their mechanism and biomedical application is underway.

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