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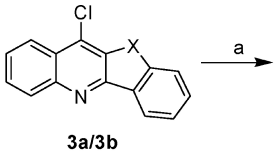
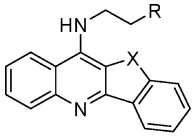


3a/3b



4

4a X = NH, R = morphoryl
4b X = NH, R = $N(CH_3)_2$
4c X = NH, R = $CH_2N(CH_3)_2$
4d X = NH, R = OH
4e X = NH, R = CH_2OH
4f X = O, R = morphoryl
4g X = O, R = $N(CH_3)_2$
4h X = O, R = $CH_2N(CH_3)_2$
4i X = O, R = OH
4j X = O, R = CH_2OH

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Synthesis and Evaluation of Quindoline Derivatives as G-Quadruplex Inducing and Stabilizing Ligands and Potential Inhibitors of Telomerase

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A new series of quindoline derivatives (**4a**−**j**) were designed and synthesized to develop novel and potent telomerase inhibitors. The interaction of the G-quadruplex of human telomere DNA with these newly designed molecules was examined via circular dichroism spectroscopy and electrophoretic mobility shift assay (EMSA). The selectivity between the quindoline derivative (**4a**) and G-quadruplex or duplex DNA was investigated by competition dialysis. These new compounds as inhibitors of telomerase were also investigated through the utilization of modified telomerase repeat amplification protocol (TRAP) assay. The results revealed that the introduction of electron-donating groups such as substituted amino groups at the C-11 position of quindoline significantly improved the inhibitory effect on telomerase activity ($^{Tel}IC_{50}$ > 138 μM for quindoline, 0.44−12.3 μM for quindoline derivatives **4a**−**j**). The quindoline derivatives not only stabilized the G-quadruplex structure but also induced the G-rich telomeric repeated DNA sequence to fold into quadruplex.

1. Introduction

Telomere is a tandemly repeated DNA sequence located at the very end of chromosomes that protects the termini of chromosomes from recombination, end to end fusion, and degradation. It has been reported that the functions and activities of telomere are closely associated with senescence replication, the cell cycle clock, and other biological processes.[1] Telomerase is a DNA polymerase containing an endogenous RNA template.[2] This enzyme adds the telomeric repeated sequences onto the ends of telomere, thus maintaining the length of telomere in tumor cells commensurate with successive cell division. Kim et al. reported that telomerase was present in about 85−90% of human cancer cells, while most of the somatic cells appeared to lack detectable levels of this enzyme.[3] This ribonucleoprotein enzyme was therefore deemed to maintain the length of the telomeres in these cells and rendered the tumor cells with an almost infinite capacity to divide and to be immortalized.[4] This unique activity of telomerase makes the enzyme an ideal probe for tumor diagnosis and a target for cancer chemotherapy.[5]

Cryptolepine (Figure 1b) is a naturally occurring indoloquinoline alkaloid. This compound and its hydrochloride salt possess interesting biological properties and have been used as antimalarial drugs in Central and Western Africa for centuries. Cryptolepine is also a rare example of a natural product whose preparation[6] was reported prior to its isolation from natural resource. Further mechanistic investigation at the molecular level demonstrated that cryptolepine could interact with DNA through intercalation[7] and consequently interfere with the catalytic activity of topoisomerase II.[8] Most recent studies further revealed that some structural derivatives of cryptolepine were capable of interacting with the G-quadruplex form of DNA (Figure 1a) and showed an inhibitory effect on telomerase.[9] To explore novel and potent telomerase inhibitors for cancer chemotherapy, we have recently designed and synthesized a new series of quindoline (Figure 1c) analogues in our laboratories and examined their interaction with G-quadruplex DNA as well as their inhibitory effect on telomerase activity.

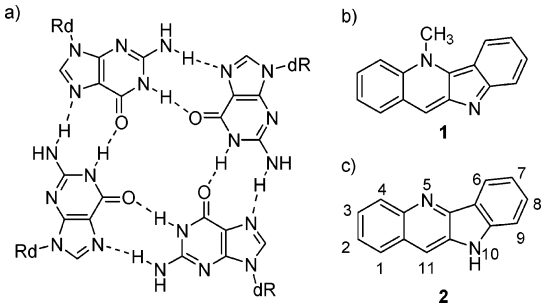

Figure 1. Structures of the G-quartet (a), cryptolepine (b), and quindoline (c).

2. Chemistry

2.1. Design of the Quindoline Derivatives. The telomeric ends of chromosomes consist of tandem repeats of simple guanine-rich DNA protein-associated motifs whose function is to protect the ends from

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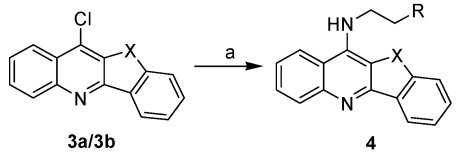
Scheme 1. Syntheses of Quindoline Derivatives **4a**−**j** and the Structures of **5** and **6**[a]



3a/3b 4

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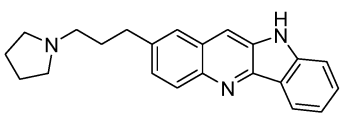
4i X = O, R = OH
4j X = O, R = CH_2OH



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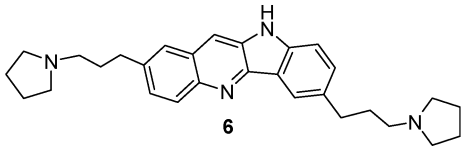

6

[a] Reaction conditions: (a) substituted alkylamine, phenol, 120 °C.

unwanted DNA damage repair, recombination, and end-fusions. Folding of telomeric DNA into a four-stranded structure involving G-quartets (Figure 1a) has been shown to inhibit the enzymes from catalyzing the synthesis of telomeric DNA repeats.[10] It is expected that synthetic molecules that induce and stabilize such G-quadruplex structures may be effective telomerase inhibitors.[11]

The crystal structure of human telomeric sequence has shown that this intramolecular quadruplex has four strands with three stacked G-quartets forming the central platform and a polarized carbonyl channel going through the stack of G-quartets. The crystal structures of quadruplex−ligand complexes[12] have revealed that ligands bind onto a G-quartet at the end of a stack, with the planar chromophore moieties participating in $\pi-\pi$ stacking interactions with the guanines and substituents lying in the grooves of the structures.

On the basis of an X-ray crystallographic study on DNA−cryptolepine interaction, Lisgarten et al. showed that the aromatic six-member ring of the indole moiety in the cryptolepine was stacked between the two cytosines of the DNA double strains, the aromatic ring of the quinoline moiety of the cryptolepine was stacked between two guanines, and the five-member ring was located in the middle.[13] Such an orientation of the cryptolepine molecule in DNA double strands indicates that the positive charge center of cryptolepine is located near the center of the axis of the DNA double helix, which facilitates the maximal electrostatic interaction of the drug molecule with the two propionate backbones of the DNA double strands. The electrostatic interaction between cryptolepine and DNA is so strong that it becomes the major factor stabilizing the DNA−cryptolepine complex.

It is expected that introducing an electron donor such as an amino group on the 11-position of quindoline may significantly increase the electron density of the 5-N atom, which may then be protonated at physiological pH, resulting in an increase of the electrostatic interaction between the quindoline derivatives and the negative electrostatic center of the G-quadruplex. On the basis of such an assumption, we prepared several quindoline derivatives **4a**−**j** (Scheme 1).

2.2. Synthesis of Quindoline Derivatives and the Measurement of the pK_a Values of Their Conjugate Acids. The synthesis of 11-chloroquindoline derivatives **3a**,**b** has been reported by Bierer et al.[6] The reaction of compounds **3a**,**b** with substituted alkylamines gave the final products of 11-aminoquindolines **4a**−**j** (Scheme 1).

Table 1. pK_a Value of the Conjugated Acid of Compounds **4a**−**j**

	quindoline	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**
pK_a (5-N−H)	4.2	8.4	8.3	8. 4	8.3	8.3	8.2	8.2	8.3	8.2	8.2

The significant increase of the electron density of the 5-N atom of compounds **4a**−**j** possessing 11- substituted amino groups was evidenced by the easy protonation of the 5-N atom of these compounds at physiological pH (7.4). The pK_a values of the conjugated acids of compounds **4a**−**j** were measured by UV spectroscopy and are summarized in Table 1.[14]

3. Results and Discussion

3.1. Inducing G-Quadruplex by Quindoline Derivatives. To investigate the effect of the 11-substituted amino groups of the quindoline derivatives on the interaction with G-quadruplex, circular dichroism (CD) spectroscopy of G-rich DNA containing the human telomeric sequence $d[G_3(T_2AG_3)_3]$ (telo21) was carried out in the presence of quindoline or quindoline derivative **4a**. Significant CD spectral change for telo21 was observed in the presence of **4a**, while little change in the presence of quindoline was observed (Figure 2A). The CD spectra of telo21 without any metal cations at room temperature exhibited a negative band at 235 nm, a small positive band at 255 nm, and a large positive band at 295 nm. These results suggested that telo21 might coexist as two types of quadruplex DNA structure: parallel and antiparallel.[15a,b] Upon the addition of **4a**, the CD spectra at ∼250 nm was suppressed, indicating a possible decrease of the parallel form of G-quadruplex DNA, while the addition of quindoline showed little effect on the CD spectra. Similarly, compounds **4b**−**j** also induced the remarkable CD changes under the same conditions (Supporting Information). These results indicated that the 11-substituted amino quindolines **4b**−**j** induced the G-rich human telomere DNA to form the G-quadruplex, which was consistent with the results of the thermodynamic stability experiments.

Further CD spectroscopic analysis was carried out to distinguish the parallel and antiparallel forms of G-quadruplex of telo21.[15−17] As shown in Figure 2B, telo21 tended to form antiparallel G-quadruplex in the presence of 0.1 M Na^+ ions (curve 1) and to form mixed parallel/antiparallel G-quadruplexes in the presence of

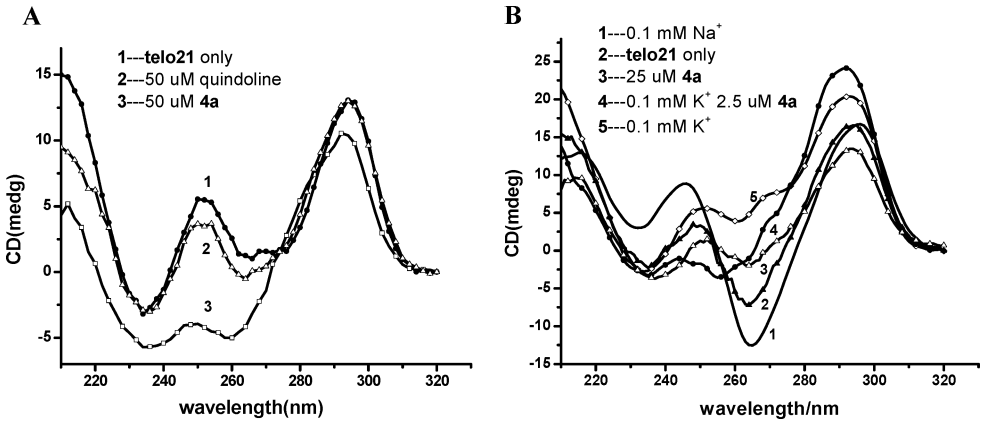

Figure 2. CD spectra of telo21 in Tris-HCl buffer, 10 mM, pH 7.4: (A) without cations or drug (●), with 50 μM **4a** (□), and with 50 μM quindoline (△); (B) with 100 mM Na^+ (−), 100 mM K^+ (◇), 100 mM K^+ and 2.5 μM **4a** (●), telo21 only (▲), 25 μM **4a** (△).

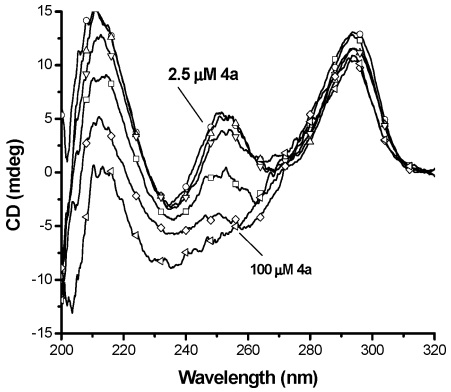

Figure 3. Change of the CD spectra of telo21 in the presence of compound **4a**. The concentrations of **4a** were 2.5 (○), 5 (△), 10 (▽), 25 (□), 50 (◇), and 100 (left facing open triangle) μM. All spectra were collected in a strand concentration of 5 μM in Tris-HCl buffer, 10 mM, pH 7.4.

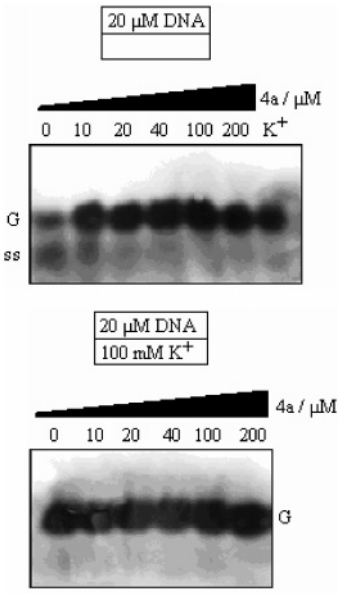

Figure 4. Effect of **4a** on the assembly of the telo21 structure illustrated by EMSA. (Top) **4a** was titrated against 20 μM telo21 in Tris-HCl buffer. Major bands were identified as G-quadruplex (G) and single-strand DNA (ss). (Bottom) Compound **4a** was titrated against 20 μM telo21 in Tris-HCl buffer containing 100 mM K^+. The major band was identified as G-quadruplex (G).

0.1 M K^+ ions (curve 5). When **4a** was added to a mixture containing 0.1 M of K^+, the signal at ~270 nm was suppressed, indicating the possible destruction of the parallel structure of the mixed G-quadruplex conformations (curve 4).

Concentration titration CD spectra of **4a** with telo21 in a Tris-HCl buffer system were recorded (Figure 3). It can be seen from the CD spectra that the peak at 295 nm remained unchanged, and the peak at 250 nm was gradually suppressed along with an increase of the concentration of **4a**. It was obvious that the concentration of **4a** had a significant effect on the conformational change of the G-quadruplex. According to our findings and those reported by Sugimoto et al.,[15] it appeared that in the presence of compound **4a**, the parallel G-quadruplex decreased instead of forming an antiparallel G-quadruplex.

EMSA (electrophoretic mobility shift assay) was performed to identify whether the quindoline derivatives were bound to the special DNA structure or induced the formation of certain secondary structures.[15b,c] The telo21 compound was incubated in the presence of increasing concentrations of **4a** alone or **4a** with 100 mM KCl. According to previous gel-shift data obtained under similar experimental conditions and with mobility standards of the G-quadruplex induced by potassium ions,[18] the major bands were identified as single-strand DNA (ss) and G-quadruplex DNA (G) (Figure 4). The results confirmed that **4a** could induce the G-rich human telomere DNA to form the G-quadruplex.

3.2. Thermodynamic Stability of the Telomeric G-Quadruplex in the Presence of Quindoline Derivatives. The binding affinity of quindoline derivatives to a human telomeric DNA sequence was studied by measuring the thermodynamic stability profile in the absence or presence of quindoline derivatives without metal ions such as K^+ or Na^+. We monitored a change of the absorption at 295 nm, which was a typical index for the conformational change from single- or double-stranded DNA to G-quadruplex DNA.[15c] The CD spectra of telo21 in the presence of **4a** (Figure 5A) showed a positive band around 295 nm at room temperature, indicating that a major fraction was an antiparallel G-quadruplex structure at room temperature. In contrast, the CD spectra of DNA showed a positive band at around 260 nm at high temperature, indicating the destuction of the G-quadruplex structure and the formation of the single-stranded structure upon heating

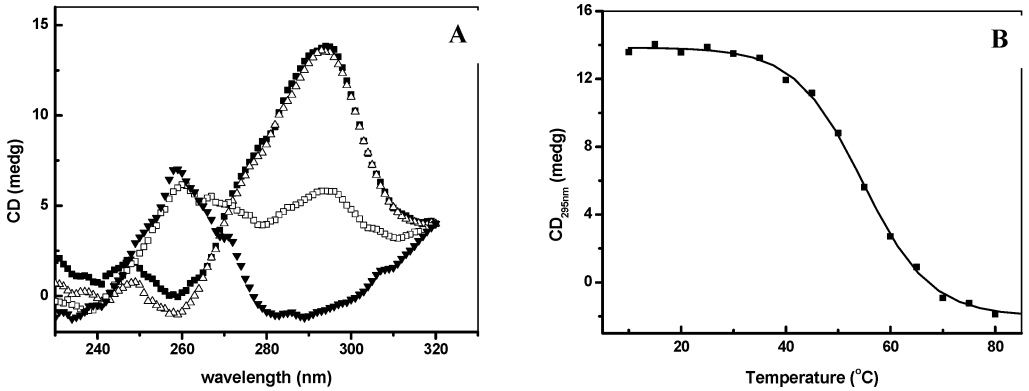

Figure 5. (A) CD spectra of telo21 with 50 μM **4a** at 10 °C (■), 25 °C (△), 55 °C (□), and 70 °C (▼). (B) CD melting profiles of telo21 with 50 μM **4a**. All spectra were collected in a strand concentration of 5 μM in Tris-HCl buffer, 10 mM, pH 7.4.

Table 2. T_m and T_m of the G-Quadruplex Induced by **4a**−**j**, **5**, and **6**[a]

compd	T_m (°C)	ΔT_m (°C)	compd	T_m (°C)	ΔT_m (°C)
4a	54.5	14.5	**4f**	54.0	14.0
4b	62.3	22.3	**4g**	58.1	18.1
4c	61.0	21.0	**4h**	56.7	16.7
4d	60.0	20.0	**4g**	58.1	18.1
4e	54.0	14.0	**4j**	53.4	13.6
5[9b]	58	5	**6**[9b]	68	15

[a] For **4a**−**j**, measured by CD spectra in 10 mM Tris-HCl buffer (pH 7.4) without metal ions. For **5** and **6**, measured by FRET in 50 mM potassium cacodylate. The melting point of native DNA quadruplex was 53 °C.

of the DNA. Figure 5B showed the normalized CD intensity of telo21 with 50 μM **4a** in a Tris-HCl buffer (pH 7.4) at 295 nm vs the temperature. The melting of native DNA quadruplex in 10 mM Tris-HCl occurred at 40.0 °C. The T_m value (54.5 °C) for the G-quadruplex structure of telo21 induced by **4a** was calculated from the CD melting curves at 295 nm using a nonlinear least-squares fitting. It was clear that **4a** could increase the melting temperature of the G-quadruplex by about 14.5 °C, indicating a high affinity of **4a** for the human telomeric sequence and resulting in increased stability of the telomeric G-quadruplex. The designed compounds had binding affinity similar to those of some reported quindoline derivatives such as **5** and **6**, based on a comparison of the ΔT_m values that were determined in the presence of K^+.[9b] The T_m and ΔT_m of the G-quadruplex induced and stabilized by **4b**−**j** were summarized in Table 2.

3.3. Competition Dialysis Assay. To evaluate the selectivity of quindoline derivative **4a** for G-quadruplex vs duplex DNA, we performed a competitive dialysis experimental using two types of G-quadruplex (telo21 and PU-18) vs duplex DNA (CT DNA and Herring sperm DNA), respectively. Higher binding affinity was reflected by the higher concentration of ligands accumulated in the dialysis tube containing the specific form of DNA.[19] The data in Figure 6 were shown as bar graphs in which the amount of bound ligand was plotted for four structurally different nucleic acids. In this assay, the various nucleic acids were dialyzed simultaneously against a certain free ligand (**4a**) solution. The amount of ligand bound was directly proportional to the binding constant for each conformational form of DNA. As shown in Figure 6, **4a** interacted preferentially with G-quadruplexes (telo21 and PU-18), whereas a weak affinity for duplex DNA (CT DNA and Herring sperm

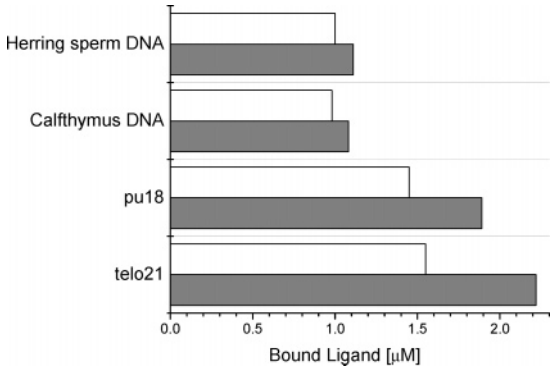

Figure 6. Results of competition dialysis experiment. A 1 μM solution of **4a** was dialyzed against four different nucleic acid structures (45 μM) for 24 h. The amount of **4a** bound to each structure was plotted as a bar graph. The SDS treating time was 3 h (white) and 48 h (gray).

DNA) was observed. When the dialysis mixtures were treated with SDS to release the binding compound, the release of **4a** in the G-quadruplex DNA was obviously slower than that in duplex DNA (Figure 6). The result indicated that the binding of compound **4a** to G-quadruplex DNA was stronger than that of **4a** to duplex DNA.

3.4. In Vitro Inhibitory Activity. The quindoline derivatives were also subjected to telomerase inhibition experiments using a modified telomerase repeat amplification protocol (TRAP) assay.[3,20] The telomerase extracts from cracked K562 cell lines were used, and the IC_{50} values of these compounds were listed in Table 3. The results clearly showed that the telomerase inhibition properties of these quindoline derivatives were significantly improved upon introduction of electron donors into the parent compound of quindoline.

It was found that the inhibitory potential of telomerase was enhanced with an increase of the charge density at 5-N (reflected by the 5-NH pK_a value). This, in turn, supported our preliminary assumption that introducing an electron donor group at the 11-position of quindoline could significantly improve its inhibitory ability against telomerase. Further study of thermal denaturation showed that replacement of the nitrogen at the 10-position with oxygen led to reduced stability of the ligand−DNA complex, along with a decrease of the inhibitory effect on telomerase. This phenomenon is probably due to the significant difference of charge between the oxygen atom of benzofuroquinoline and the imino group (NH) of quindoline, resulting in a different

Table 3. Inhibition of Quindoline and **4a**−**j** on Telomerase

	quindoline	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**
$^{Tel}IC_{50}$ (μM)	>138	0.55	1.12	0.44	2.35	1.55	4.38	7.11	3.29	12.3	8.12

binding affinity to G-quadruplex. Furthermore, the 11-substituted quindoline derivatives with the same aromatic core but different side chains demonstrated different abilities in inducing and stabilizing the telomeric G-quadruplex structures. Obviously, the electrostatic interactions between the ligand side chains and telomeric G-quadruplex structures play an important role in their recognition of each other.

4. Conclusions

Several quindoline derivatives (**4b**−**j**) were designed and synthesized, and their interaction with the telomeric G-quadruplex has been studied. Introducing an electron-donating group such as a substituted amino group to the 11-position of quindoline significantly increased the electron density at the 5-N atom, resulting in easy protonation under physiological pH. EMSA and CD spectra studies clearly showed that these compounds were capable of inducing the formation and stabilization of the G-quadruplex and thus exhibited inhibitory effect on the telomerase. The competition dialysis experiment showed that **4a** interacted preferentially with G-quadruplexes and had comparatively weak affinity for duplex DNA. The inhibitory effect of these compounds on telomerase was also investigated through the utilization of a modified telomerase repeat amplification protocol (TRAP) assay. This study suggested that the quindoline derivatives might be potential lead compounds for the development of new telomerase inhibitors.

5. Experimental Section

Synthetic Chemistry. Melting points were recorded on a Leica Galen III hot-stage melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a 500 MHz Varian Unity INOVA spectrometer or a 300 MHz Mercury-Plus spectrometer using TMS as an internal standard in DMSO-d_6, $CDCl_3$, CD_3OD. Mass spectra were recorded on either a VG ZAB-HS (fast atom bombardment) or a Finnigan LCQ Deca XP (Electrospray) instrument. High-resolution mass spectra were obtained with a GCT-TOF. Elemental analyses were carried out on an Elementar Vario EL CHNS elemental analyzer. All compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates.

11-Chloro-10*H*-indolo[3,2-*b*]quinoline (3a). Compound **3a** was synthesized by using a literature procedure.[6b] Mp 219−221 °C (219−221 °C in ref 6c). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.86 (brs, NH), 8.37 (d, 1H, J = 7.8 Hz), 8.28 (t, 2H, J = 8.4 Hz), 7.80−7.62 (m, 4H), 7.35 (t, 1H, J = 7.5 Hz). HRMS-EI *m/z*: calcd for $C_{15}H_9N_2Cl$, 252.0454; found, 252.0453.

11-Chlorobenzofuro[3,2-*b*]quinoline (3b). Compound **3b** was synthesized by a literature procedure.[6d] Mp 158−160 °C (157−158 °C in ref 6c). FAB-MS *m/z*: 254 ($M^+ + 1$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.35 (d, 1H, J = 8.0 Hz), 8.22 (d, 1H, J = 8.0 Hz), 7.80 (d, 1H, J = 8.0 Hz), 7.75 (d, 2H, J = 8.5 Hz), 7.70 (td, 1H, J = 8.0, 7.0, 1.5 Hz), 7.51 (td, 1H, J = 8.0, 7.0, 1.5 Hz), 7.38 (m, 1H).

General Method for the Preparation of the 11-Aminoquindoline Derivatives (4). A mixture of **3** (5 mmol) and 10 g of phenol was heated at 60 °C for half an hour, then 10 mmol of *N*,*N*-dialkylalkylamine was added and the mixture was heated at 120 °C for 4 h. The mixture was cooled to room temperature and was poured to 50 mL of water. The pH of the mixture was adjusted to 10 with a 40% NaOH solution, and the organic product was extracted with chloroform, washed with saturated NaCl solution and water, and then dried with Na_2SO_4. After concentration, the product was purified by column chromatography on silicon gel. Further purification was carried out by recrystallization.

***N*′-(10*H*-Indolo[3,2-*b*]quinolin-11-yl)-(2-morpholin-4-ylethyl)amine (4a)**. Mp 208−210 °C. ESI-MS *m/z*: 347 (M^+). ^{1}H NMR (300 MHz, acetone-d_6): δ 8.45 (d, 1H, J = 8.1 Hz), 8.24 (d, 1H, J = 8.7 Hz), 8.16 (d, 1H, J = 8.4 Hz), 7.59 (t, 2H, J = 8.7 Hz), 7. 53 (t, 1H, J = 7.2 Hz), 7.43 (t, 1H, J = 7.5 Hz), 7.22 (t, 1H, J = 7.5 Hz), 4.07 (t, 2H, J = 5.7 Hz), 3.68 (t, 4H, J = 4.2 Hz), 2.84 (t, 2H, J = 5.7 Hz), 2.63 (m, 4H). Anal. Calcd for $C_{21}H_{22}N_4O$: C, 72.81; H, 6.40; N, 16.17. Found: C, 72.69; H, 6.52; N, 16.24.

***N*′-(10*H*-Indolo[3,2-*b*]quinolin-11-yl)-*N*,*N*-dimethylethane-1,2-diamine (4b).** Mp 189−191 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.06 (s, 1H), 10.80 (s, 1H), 9.20 (s, 1H), 8.60 (d, 1H, J = 8.7 Hz), 8.70 (d, 1H, J = 7.5 Hz), 8.28 (d, 1H, J = 8.1 Hz), 7.89 (d, 2 H, J = 7.8 Hz), 7.67 (t, 1H, J = 7.5 Hz), 7.57 (t, 1H, J = 7.5 Hz), 7.32 (t, 1H, J = 7.5 Hz), 4.60 (m, 2H), 3.63 (t, 2H, J = 5.4 Hz), 2.93(s, 6H). HRMS-EI *m/z*: calcd for $C_{19}H_{20}N_4$, 304.1688; found, 304.1680. Anal. Calcd for $C_{19}H_{20}N_4$: C, 74.97; H, 6.62; N, 18.41. Found: C, 74.92; H, 6.76; N, 18.50.

***N*′-(10*H*-Indolo[3,2-*b*]quinolin-11-yl)-*N*,*N*-dimethylpropane-1,3-diamine (4c).** Mp 197−199 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 8.37 (d, J = 7.8 Hz 1 H), 8.18 (d, J = 7.8 Hz 1 H), 8.03 (d, J = 8.7 Hz 1 H), 7.54 (m H), 7.41 (m 3 H), 7.19 (t, J = 6.6 Hz 1 H), 3.89 (t, J = 6.3 Hz 2 H), 2.58 (t, J = 6.3 Hz 2 H), 2.31 (s, 6 H), 1.93 (m, 2 H). HRMS-EI *m/z*: calcd for $C_{20}H_{22}N_4$, 318.1844; found, 318.1847. Anal. Calcd for $C_{20}H_{22}N_4$: C, 75.44; H, 6.96; N, 17.60. Found: C, 75.30; H, 7.15; N, 17.50.

***N*′-(10*H*-Indolo[3,2-*b*]quinolin-11-ylamino)ethanol (4d).** Mp 227−229 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.58 (d, 1H, J = 8.7 Hz), 8.36 (d, 1H, J = 7.5 Hz), 8.19 (d, 1H, J = 7.2 Hz), 7.89 (t, 1H, J = 7.2 Hz), 7.77−7.58 (m, 3H), 7.34 (t, 1H, J = 6.9 Hz), 4.30 (s, 1H, OH), 4.15 (s, 2H), 3.87 (s, 2H). HRMS-EI *m/z*: calcd for $C_{17}H_{15}N_3O$, 277.1215; found, 277.1178. Anal. Calcd for $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45; N, 15.15. Found: C, 73.49; H, 5.67; N, 15.24.

***N*′-(10*H*-Indolo[3,2-*b*]quinolin-11-ylamino)propanol (4e).** Mp 232 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.77 (s, 1H, NH), 8.90 (s, 1H), 8.65 (d, 1H, J = 5.4 Hz), 8.56 (d, 1H, J = 4.8 Hz), 8.16 (d, 1H, J = 5.1 Hz), 7.90 (t, 1H, J = 4.8 Hz) 7.75 (d, 1H, J = 5.1 Hz), 7.69 (t, 1H, J = 4.5 Hz), 7.60 (t, 1H, J = 4.5 Hz), 7.36 (t, 1H, J = 4.8 Hz), 5.18 (s, 1H, OH), 4.19 (t, 2H, J = 3.9 Hz, J = 3.9 Hz), 3.68 (t, 2H, J = 3.3 Hz), 2.04 (m, 2H). HRMS−FAB *m/z*: calcd for $C_{18}H_{17}N_3O$, 291.1372; found, 291.1360. Anal. Calcd for $C_{18}H_{17}N_3O$: C, 74.20; H, 5.88; N, 14.42. Found: C, 74.06; H, 6.01; N, 14.31.

(10*H*-Benzofuro[3,2-*b*]quinolin-11-yl)-(2-morpholin-4-ylethyl)amine (4f). Mp 216−218 °C. ESI-MS *m/z*: 348 (M^+). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.32 (d, 1H, J = 8.5 Hz), 8.20 (d, 1H, J = 7.5 Hz), 7.99 (d, 1H, J = 8.5 Hz), 7.72 (d, 1H, J = 8.0 Hz), 7.64−7.69 (m, 2H), 7.45−7.49 (m, 2H), 7.22 (brs, 1H), 4.05 (t, 2H, J = 10.5 Hz, J = 6.5 Hz), 3.59 (s, 4H), 2.74 (brs, 2H), 2.54 (s, 4 H). HRMS-EI *m/z*: calcd for $C_{16}H_{11}N_2O$ [$M^+ - O(C_2H_4)_2NCH_2$], 247.0871; found, 247.0839. Anal. Calcd for $C_{21}H_{21}N_3O_2$: C, 72.60; H, 6.09; N, 12.10. Found: C, 72.45; H, 6.21; N, 11.95.

(10*H*-Benzofuro[3,2-*b*]quinolin-11-yl)-*N*,*N*-dimethylethane-1,2-diamine (4g). Mp 208−210 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.30 (d, 1H, J = 8.5 Hz), 8.19 (dt, 1H, J = 7.0, 1.0 Hz), 7.99 (dd, 1H, J = 8.5, 1.0 Hz), 7.63−7.71 (m, 3H), 7.46 (tt, 1H, J = 8.0, 2.0 Hz), 7.13 (t, 1H, J = 6.0 Hz), 4.02 (q, 2 H, J = 6.5 Hz), 2.66 (t, 2H, J = 6.5 Hz), 2.28 (s, 6H). HRMS-EI *m/z*: calcd for $C_{19}H_{19}N_3O$, 305.1528; found, 305.1518. Anal. Calcd for $C_{19}H_{19}N_3O$: C, 74.73; H, 6.27; N, 13.76. Found: C, 74.56; H, 6.38; N, 13.51.

(10*H*-Benzofuro[3,2-*b*]quinolin-11-yl)-*N*,*N*-dimethylpropane-1,2-diamine (4h). Mp 230 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.30 (d, 1H, J = 8.5 Hz), 8.20 (d, 1H, J = 7.0 Hz), 8.00 (d, 1H, J = 7.5 Hz), 7.70 (d, 1H, J = 8.5 Hz), 7.65 (q, 2H, J = 8.5 Hz), 7.45 (t, 3H, J = 7.0 Hz), 3.97 (q, 2H, J = 7.5 Hz, J = 6.0 Hz), 2.46 (t, 2H, J = 7.0 Hz), 2.21 (s, 6H), 1.90 (m, 2H). HRMS-EI *m/z*: calcd for $C_{20}H_{21}N_3O$, 319.1685; found, 319.1660. Anal. Calcd for $C_{20}H_{21}N_3O$: C, 75.21; H, 6.63; N, 13.16. Found: C, 75.04; H, 6.48; N, 12.95.

(10*H*-Benzofuro[3,2-*b*]quinolin-11-ylamino)ethanol (4i). Mp 216−218 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.24 (s, 1H), 8.38 (d, 1H, J = 8.0 Hz), 8.20 (d, 1H, J = 8.0 Hz), 7.98 (d, 1H, J = 8.0 Hz), 7.72 (d, 1H, J = 8.0 Hz), 7.65 (m, 2H), 7.45 (t, 2H, J = 8.0 Hz), 4.80 (t, 1H, J = 5.5 *Hz*), 4.02 (q, 2H, J = 6.0 Hz), 3.76 (q, 2H, J = 6.0 Hz). HRMS-EI *m/z*: calcd for $C_{17}H_{14}N_2O_2$, 278.1055; found, 278.1053. Anal. Calcd for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.14; H, 5.21; N, 9.95.

(10*H*-Benzofuro[3,2-*b*]quinolin-11-ylamino)propanol (4j). Mp 206 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 8.30 (d, 1H, J = 7.5 Hz), 8.17 (d, 1H, J = 8.4 Hz), 8.00 (d, 1H, J = 8.4 Hz), 7.65 (m, 3H), 7.45 (m, 2H), 4.15 (t, 2 H, J = 6.9 Hz), 3.83 (t, 2H, J = 6.0 Hz), 2.09 (m, 2H). HRMS-EI *m/z*: calcd for $C_{18}H_{16}N_2O_2$, 292.1212; found, 292.1216. Anal. Calcd for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.52; N, 9.58. Found: C, 73.74; H, 5.75; N, 9.33.

Determination of pK_a Values.[14] The pK_a values of the quindoline derivatives were measured by using the method reported by Irvin[14a] and Knight.[14b] A series of solutions of quindoline derivatives with different pH values were prepared. The molar extinction coefficient of each solution was determined at the two wavelengths at which ϵ showed a bigger change in going from acidic to basic solution. The ϵ values were plotted against the pH. The pH that fell on the curve midway between the horizontal sections was taken as pK_a.

Assay of the Interaction of Small Molecules with DNA Using Circular Dichroism Spectroscopy. A 21-mer oligonucleotide d($G_3(T_2AG_3)$)$_3$ at a final concentration of 5 μM was resuspended in Tris-HCl buffer (10 mM, pH 7.4) containing the specific cations and the molecule to be tested. The sample was heated to 90 °C, then gradually cooled to room temperature, and was incubated at 4 °C for several hours. The CD spectra were recorded on a Jasco J-810 spectropolarimeter at 320−200 nm, using 16 scans at 100 nm/min, 1 s response time, and 1 nm bandwidth. Cuvettes of 1 mm width with black quartz sides to mask the light beam were used for the measurements. A buffer baseline was collected in the same cuvette and subtracted from the sample spectra. The CD spectra were obtained by taking the average of at least three scans made from 200 to 350 nm. The final spectra were normalized to have zero ellipticity at 320 nm. In the melting studies, the temperature of the sample was maintained by a Julabo HD-25 temperature controller. The melting curves of the G-quadruplex were measured with the intensity at 295 nm. Before the CD spectroscopy, all the samples were thermally treated as described above. The heating rate was 1.0 °C min^{-1}.

EMSA (Electrophoretic Mobility Shift Assay).[18] The oligomer at 20 μM was heated to 95 °C for 10 min in Tris-HCl buffer (10 mM, pH 7.4) containing 100 mM KCl. After the DNA was cooled to room temperature, a 2 μL stock solution of **4a** was added to each sample to produce the specified concentrations at a total volume of 20 μL. The reaction mixture was incubated for 4−8 h at room temperature. After incubation, 4 μL of loading buffer (50% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanol) was added to each mixture. Twenty microliter aliquots of each sample were subsequently analyzed by native 15% PAGE (the gel was prerun for 30 min). Electrophoresis proceeded for 2 h in TBE running buffer. The gels were silver-stained following a previously reported protocol.[21]

Modified Telomeric Repeat Amplification Protocol (TRAP) Assaay.[20] The ability of agents to inhibit telomerase in a cell-free assay was assessed with a modified TRAP assay using extracts from exponentially growing A2780 human ovarian carcinoma cells as described previously. The TRAP assay was performed in two steps: (a) telomerase-mediated extension of the forward primer (TS: 5′-AATCCGTCGAGCAGAGTT, Oswel Ltd., Southampton, U.K.) contained in a 40 μL reaction mixture comprising TRAP buffer (20 mM, pH 8.3), 68 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 0.05% v/v Tween-20, 0.05 μg of bovine serum albumin, 50 μM of each deoxynucleotide triphosphate, 0.1 μg of TS primer, and 3 μCi of [R-32P]dCTP (Amersham plc, U.K.). The protein (0.04 μg) was incubated with the reaction mixture ± agent (acid addition and quaternary dimethiodide salts) at final concentrations of up to 50 μM for 20 min at 25 °C. A lysis buffer (no protein) control, heat-inactivated protein control, and 50% protein (0.02 μg) control were included in each assay. (b) While the mixture was being heated at 80 °C in a polymerase chain reaction (PCR) block of a thermal cycler (Hybaid, U.K.) for 5 min to inactivate telomerase activity, 0.1 μg of reverse CX primer (3-AATCCCATTCCCATTCCCATTCCC-5) and 2 units of Taq DNA polymerase ("red hot", Advanced Biotechnologies) were added. A three-step PCR was then performed: 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min for 31 cycles. Telomerase-extended PCR products in the presence or absence of compounds were determined either by electrophoretic separation using 8% w/w acrylamide denaturing gels and analysis by phosphorimaging or autoradiography or by harvesting on Whatman filters (25-mm glass microfiber) and analysis by liquid scintillation counting.

Competition Dialysis Experiment. A Tris-HCl buffer (10 mM, pH 7.4) containing 100 mM NaCl was used for all experiments. For each competition dialysis assay, 400 mL of dialysate solution containing 1 μM compound **4a** was placed into a beaker. A volume of 0.5 mL (at 45 μM monomeric unit) of each of the DNA samples was pipeted into a separate 0.5 mL dialysis cassette (Pierce). The DNA samples included telo21 (partial sequence in human telomere that may form the G-quadruplex structure), whose sequence was 5′-GGGTTAGGGTTAGGGTTAGGG-3′; PU-18 (partial sequence in c-myc gene that may form the G-quadruplex structure), whose sequence was 5′-AGGGTGGGGAGGGTGGGG-3′; and duplex DNA Herring sperm DNA and Calfthymus DNA. The entire dialysis cassettes were then placed in the beaker containing the dialysate solution. The contents were allowed to equilibrate with continuous stirring for 24 h at room temperature. At the end of the equilibration period, DNA samples were carefully removed to microfuge tubes and were taken to a final concentration of 1% (w/v) sodium dodecyl sulfate (SDS). The total concentration of **4a** (C_t) within each dialysis cassette was then determined spectrophotometrically using a wavelength of 415 nm and an extinction coefficient of 2.325 M^{-1} cm^{-1}. The SDS treating time was 3 and 48 h. The free ligand concentration (C_f) was determined spectrophotometrically using an aliquot of the dialysate solution. The amount of bound **4a** was determined by the difference between the total ligand concentration and the free ligand concentration ($C_b = C_t - C_f$).

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Supporting Information Available: CD spectra at room temperature for compounds **4b**−**j**, LRMS, HRMS, and ^{1}H NMR spectra of compounds **3a**,**b** and **4a**−**j**. This material is available free of charge via the Internet at http://pubs.acs.org.

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