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Synthesis and evaluation of cationic phthalocyanine derivatives as potential inhibitors of telomerase

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Abstract—A series of water-soluble cationic phthalocyanine derivatives (**1**–**10**) were designed and synthesized to develop novel and potent telomerase inhibitors. These phthalocyanine derivatives as inhibitors of telomerase were investigated via modified telomerase repeat amplification protocol (TRAP) assay. The TRAP assay indicates that these cationic compounds had strong telomerase inhibitory activity ($IC_{50} < 1.65$ μM). To determine whether the phthalocyanine derivatives binding to G-quadruplex enhance the block to DNA synthesis, primer extension reactions were carried out in the presence of phthalocyanines. The interaction of the G-quadruplex of telomerase DNA with these molecules was examined by CD melting and PCR stop assay. These cationic phthalocyanine derivatives can stabilize G-quadruplex, which is demonstrated by the increased T_m values. All these results indicate that the phthalocyanine derivatives might be potential lead compounds for the development of new telomerase inhibitor.

1. Introduction

Telomeres are the guanine-rich and simple repeat sequences of TTAGGG. They constitute the physical termini of eukaryotic chromosomes. Maintenance of telomeres is known as telomerase mediates and is significant for immortalization in cancer cells.[1] Most cancer cells have increased levels of telomerase, whereas telomerase activity is low in human somatic cell.[2] Therefore, this special property could be a potential target for antitumor drugs.[3] The Human telomeric DNA contains double-stranded d(TTAGGG/CCCTAA) repeats and a single-stranded 3′ overhang. This overhang may fold into intramolecular G-quadruplex structures. If the formation of G-quartets happened, it could directly inhibit telomerase and stop replication of DNA. So, the stabilization of quadruplex structure by small molecules could inhibit telomerase.[4,5]

Phthalocyanine is a large conjugation with many electrons. They have wide application in many fields.[6] Recently, Sanders' group first reported that tetramethylpyridiumporphyrazines could stabilize and induce G-quadruplex.[7] Our group previously reported an octa-cationic quaternary ammonium zinc phthalocyanine and found that it could be a potent G-quadruplex stabilizer and telomerase inhibitor. During our experiments, we found that it could increase polymerase pausing in the TRAP assay by stabilizing the G-quadruplex structure formed in low K^+ concentration buffer. Interestingly, we also found that it could induce intramolecular G-quadruplex structure transition from the antiparallel to parallel form and induce the parallel structure formation in cation-deficient condition.[8] To further explore novel and potent telomerase inhibitors for cancer chemotherapy, a series of phthalocyanines were designed and synthesized in our laboratories to examine their interaction with G-quadruplex DNA as well as their inhibitory action on telomerase activity.

A disadvantage of both metal-free phthalocyanine and metal phthalocyanine is their limited solubility in water. It is possible to attach a wide variety of substituents at the periphery of the dinitrile to improve their water solubility. Soluble phthalocyanines are obtained by peripheral cationic substitution or anionic substitution with sulfur and oxygen. In this paper, we choose metal phthalocyanines and metal-free phthalocyanines with quaternary ammonium group (Scheme 1) on the periphery to change the solubility in water to study their activities of inhibition on telomerase. Their capabilities to inhibit

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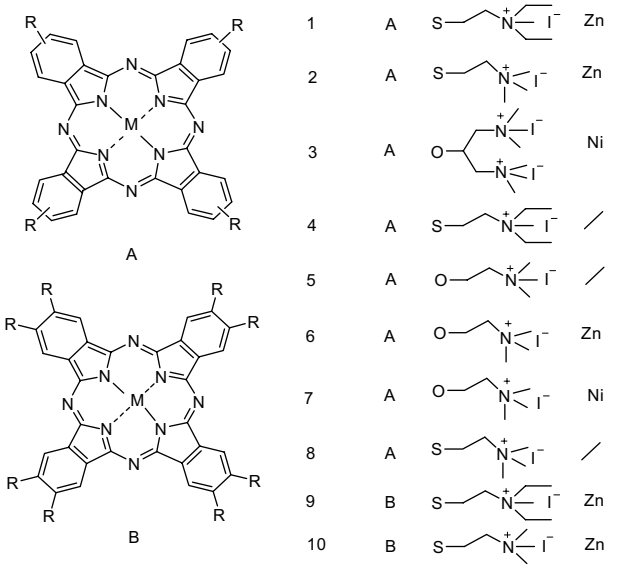


Scheme 1. Chemical formulae of the molecules studied.

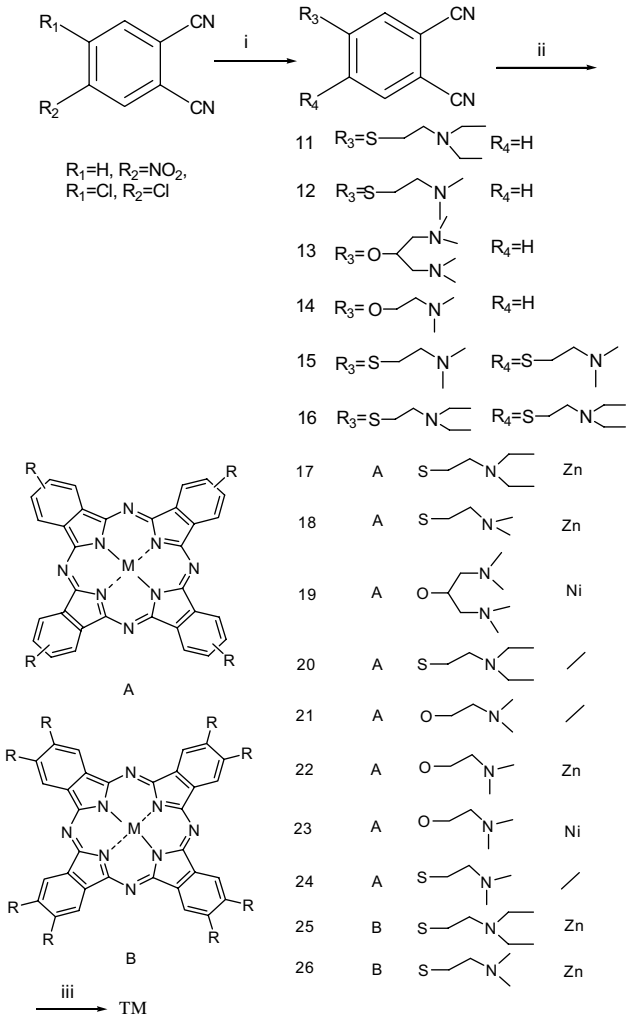
telomerase were measured by TRAP assay. The abilities of stabilizing G-quadruplex were measured by polymerase stop assay and their selectivities to stabilize G4 were measured by CD melting.

2. Result and discussion

2.1. Chemistry

In order to investigate the structure–activity relationship, we synthesized a series of phthalocyanines including zinc, nickel, and free phthalocyanine to study their effect of different cores. To evaluate the effect of substitution, we synthesized phthalocyanines with different substituents containing oxygen and sulfur at the periphery. We propose that substituents with oxygen will increase their ability to form hydrogen bonding. Also, different charge number might play an important role in their interactions with DNA. Therefore, cationic phthalocyanines with four or eight positive charges have been synthesized.

The synthetic route is shown in Scheme 2. Compounds **11**–**16** [9–14] were prepared by a base catalyzed nucleophilicaromatic displacement of dicyanobenzene derivatives. This reaction has been used in the preparation of a variety of phthalonitriles substituted with ether or thioether. Peripheral dimethylaminoethyl or diethylamino-ethyl substituents of titanylphthalocyanines **17**–**26** are suitable for conversion into quaternary ammonium groups and this can increase products' solubility in water. Metal phthalocyanines were obtained in the presence of metal salts and phthalonitriles. Metal free phthalocyanine was obtained directly by refluxing phthalonitrile in DBU and *n*-pentanol.[15–20] Compounds **1**–**10** were obtained by methylation of compounds **17**–**26** in $CHCl_3$ in good yields. All new compounds were fully characterized by NMR, UV, TG, elemental analysis or HRMS.



Scheme 2. Synthetic pathway for the synthesis of phthalocyanines **1**–**10**. Reagents and conditions: (i) DMF, K_2CO_3/2-(diethylamino) ethanethiol hydrochloride/2-(dimethylamino) ethanethiol hydrochloride/1,3-bis(dimethyl amino)-2-propanol/2-(dimethylamino) ethanol hydrochloride; (ii) DBU, *n*-$CH_3(CH_2)_4OH$, $Zn(OAC)_2{\cdot}4H_2O$/$Ni(OAC)_2{\cdot}2H_2O$ reflux; (iii) CH_3I, $CHCl_3$, reflux.

2.2. Biological result and discussion

2.2.1. In vitro inhibitory activity. The inhibition abilities of phthalocyanines were determined by a modified telomerase repeat amplification protocol assay (TRAP). Modified TRAP is a powerful technique and could give us some information about small molecules inhibiting telomere elongation qualitatively and quantitatively.[21] In this assay, the elongated products were further amplified by PCR and visualized by dye staining. The amplification of the internal control (ITAS) is a key signal for inhibition of telomerase rather than the PCR.[22] The IC_{50} values of these compounds are listed in Table 1. The results suggested that the telomerase inhibitory abilities of phthalocyanines were potent. Some of the compounds showed high activity with IC_{50} value in the region of nanomolar. Especially the IC_{50} value of nickel phthalocyanines (compounds **3** and **7**) were as low as 20 and 32 nM (Fig. 1), respectively. It is probably due to a combination of steric and electrostatic factors. The ste-

Table 1. Inhibition of compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10** on telomerase

Compound	**3**	**4**	**6**	**7**	**8**
IC_{50} (nM)	20	213	108	32	260
Standard deviation	4.2	20	69	3.1	10
Compound	**1**	**2**	**5**	**9**	**10**
IC_{50} (μM)	1.65	1.1	0.49	0.48	0.62
Standard deviation	0.016	0.08	0.069	0.009	0.04

ric structure of Ni^{2+} complexes made themselves have more favorable to bind quadruplex and finally might lead them to have potent abilities to inhibit telomerase.[23]

Different factors, such as the number of positive charges, the substituents at the periphery, and the metals of the phthalocyanine, were taken into account in designing our compounds. From our current results, we can generally get that the activities of nickel phthalocyanines were the best. The inhibitory concentrations of compounds **1**, **2** were much higher than others. Comparing compounds **1** and **9**, **2** and **10**, **7** and **3**, we found that the activities of compounds **9**, **10**, **3** with eight positive charges are better than thoseof the corresponding compounds **1**, **2**, **7** with four positive charges. It suggests that the activity of telomerase is inhibited more obviously as the positive charges on the compounds increase, which is probably due to the fact that more positive charge enhances the binding affinities of the compounds to DNA. According to these data, the substituents at the periphery of the phthalocyanine also affect their inhibitory activities. When the core of the phthalocyanine is zinc, the activity of compounds **2** and **6** (IC_{50} of compound **2** > IC_{50} of compound **6**) demonstrated that the phthalocyanine with oxygen substituents is better than the phthalocyanine with sulfur substituents. However, the free phthalocyanine is not obeying the rule. Comparing compound **8** with compound **5**, the phthalocyanine with sulfur substituent and free phthalocyanine is better than the free phthalocyanines with oxygen substituents. We also found that the phthalocyanine with sulfur substituents could inhibit the internal control slightly (see supporting information Fig. S1). This might be attributed to hydrogen bond formed by oxygen substituents and the electric effects of different substituents. Interestingly, according to the comparison of compounds **5**, **6**, **7**, we found that phthalocyanines with oxygen substituents and different core metals have diverse inhibitory activities. With oxygen substituents on phthalocyanines, the results indicated that the effect of the core atoms of phthalocyanines on the activity is of the order Ni > Zn > H. Comparison of compounds **1** and **4**, **2** and **8** indicates that the inhibition activities of the telomerase of compounds **4**, **8** are better than those of **1**, **2**. With sulfur substituents on phthalocyanines, metal free phthalocyanine has better activity than zinc phthalocyanine, which is contrary to the results for phthalocyanine with oxygen substituents. According to the results of TRAP assay, we could conclude that the phthalocyanines with different substituents all have inhibitory activities in vitro. Their inhibitory activities of telomerase are associated with the metals of the core, substituents, and positive charges.

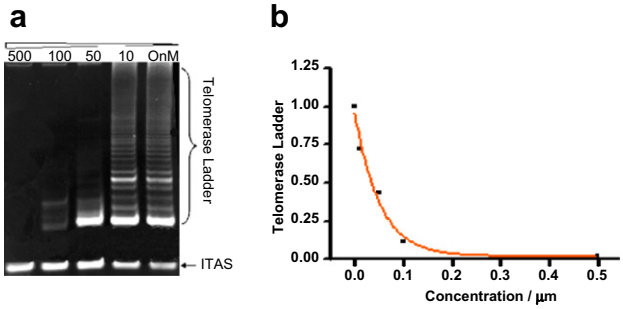


Figure 1. (a) Inhibitory activity of compound **7** on telomerase. Increasing concentrations of **7** (10–500 nM) were added in a TRAP assay containing an internal standard (ITAS). The gel shows that at the concentration range only the telomere ladder is affected; (b) Graphical determination of the IC_{50} for compound **7**.

Comparing the ability of inhibition of telomerase, phthalocyanine derivatives are better than TMPyP4. TMPyP4 showed lower activity with IC_{50} value of 1.97 μM (see Figs. S1D and S1E).

2.2.2. Polymerase stop assay. To get the information about the ability of phthalocyanine to stabilize quadruplex, a Taq DNA polymerase stop assay was used. This assay has been used to demonstrate the drug-induced stability of quadruplexes formed in the template strand.[24,25] Our experiments (Fig. 2) suggested that in the absence of phthalocyanines, with low K^+ concentration, there was only a slight pausing of DNA extension at the G-rich site. Adding phthalocyanines to the assay resulted in an enhancement of pausing that was proportional to the amount of compounds added. Figure 2 shows the block of Taq polymerase DNA synthesis by compound **6**, which indicated the ligand enhanced pausing in a concentration-dependent manner. Other phthalocyanines had the same properties. From our results (see supporting information Fig. S2), all the phthalocyanines could effectively enhance the pausing site

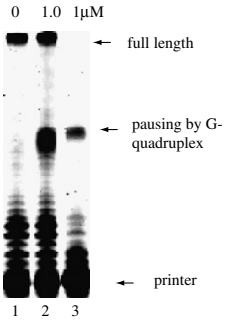


Figure 2. Concentration-dependent block of Taq polymerase DNA synthesis by the G-quadruplex structure formed on the HT4 template at 55 °C. Lane 1, control. Lanes 2, 3, the concentration of compound **6**, 0.1 and 1.0 μM, respectively. The sequencing gel shows the enhanced DNA synthesis pausing at the G-quadruplex site with increasing concentrations of compounds, arrows indicate the positions of the full-length product of DNA synthesis, the G-quadruplex pausing site, and the free primer.

from gel. Therefore, phthalocyanines could stabilize the G-quadruplex structure by observing the polymerase pausing in the buffer with low K^+ concentration.

2.2.3. PCR stop. To clarify if the stabilization of G-quadruplex structures by specific ligands was involved in the inhibition of DNA polymerase, a reported PCR-stop assay technique was used (Fig. 3). A test oligonucleotide (5′-GGGTTAGGGTTAGGGTTAGGG-3′, **Seq1**) and a complementary oligonucleotide (5′-TCTCG TCTTCCCTAA-3′, **Seq2**) could partially hybridize to the last G-repeat of the **Seq1**.[26,27] The induction of G-quadruplex formation in **Seq1** by phthalocyanine derivatives could be tested by PCR stop assay. If the ligands bind to the intramolecular G-quadruplex structures and stabilize them, the action of the DNA polymerase will be inhibited. Our results indicated that the cationic phthalocyanine can stabilize G quadruplex structure and cause PCR inhibition efficiently. The parallel experiments were performed with an oligonucleotide (5′-GG GTTAGAATTAGGGTTAGGG-3′, **Seq3**) which was designed as mutations in the guanine repeat and this will lead to inablity to form a quadruplex. So our results here showed that the phthalocyanine can selectively inhibit **Seq1** PCR stop assay through stabilizing G-quadruplex instead of inhibiting the Taq polymerase, which was also demonstrated in the polymerase stop assay (see supporting information Fig. S3). We also did the parallel experiments using TMPyP4 and found that TMPyP4 could not selectively inhibit the **Seq1** PCR stop assay. From Figure 4a and b, at the concentration of the 1.5 μM, TMPyP4 not only inhibits the **Seq1** PCR stop assay but also inhibits the **Seq3** PCR stop assay.

Meanwhile, different concentrations of phthalocyanine **6** at 0.1, 0.25, 0.5, and 1.0 μM were tested in this assay. Compound **6** showed an inhibitory effect on the hybridization of oligomer **Seq1** and **Seq2** when the derivative concentration was 1.0 μM (Fig. 4c). At the concentration of 1.0 μM the hybridization of oligomer **Seq3** and **Seq2** couldn not be inhibited completely by the compound **6** (see Fig. 4d).

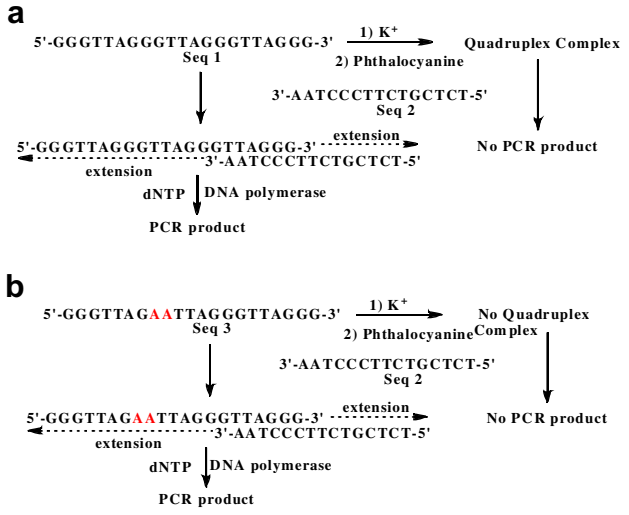


Figure 3. Principle of the PCR stop assay.[26,27] **Seq1** or **Seq3** was amplified with **Seq2** overlapping the last G-repeat. Taq polymerase extension produced a final PCR product (a and b). If G-quadruplex structure can be stabilized, then Taq polymerase extension could be inhibited. Marked bases in red correspond to the mutations that replace.

2.2.4. Thermodynamic stability of the telomeric G-quadruplex in the presence of phthalocyanines. The ability of phthalocyanine to stabilize G-quadruplex DNA was also investigated using CD melting method. As the absorbance changes at this wavelength can precisely monitor intramolecular G-quadruplex formation and dissociation, CD absorbance was monitored at 295 nm.[28] The G-quadruplex structure of G4-A was determined by CD spectra (see Fig. 5). Experiments for the stability of G-quadruplex in Na^+ buffer [29,30] with the absence and presence of phthalocyanines were performed. The melting of native DNA quadruplex in 10 mM Tris–HCl, 100 mM NaCl occurred at 43.0 °C.

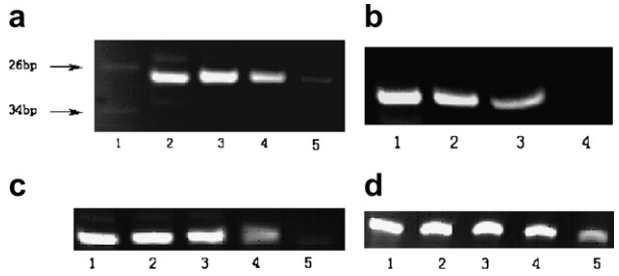


Figure 4. Effect of TMP_yP_4 and phthalocyanine **6** on the formation of the PCR-stop assay with the hybridization of G-quadruplex forming **Seq1** oligomer (a and c) and **Seq2** or with the control hybridization mutated **Seq3** oligomer (b and d) and **Seq2**. (a) Lane 1 marker, Lane 2 control, lane 3 TMP_yP_4 (0.5 μM), lane 4 TMP_yP_4 (1.0 μM), lane 5 TMP_yP_4 (1.5 μM); (b) Lane 1 control, lane 2 TMP_yP_4 (0.5 μM), lane 3 TMP_yP_4 (1.0 μM), lane 4 TMP_yP_4 (1.5 μM); (c) Lane 1 control, lane 2 phthalocyanine **6** (0.1 μM), lane 3 phthalocyanine **6** (0.25 μM), lane 4 phthalocyanine **6** (0.5 μM), lane 5 phthalocyanine **6** (1 μM); (d) Lane 1 control, lane 2 phthalocyanine **6** (0.1 μM), lane 3 phthalocyanine **6** (0.25 μM), lane 4 phthalocyanine **6** (0.5 μM), lane 5 phthalocyanine 6 (1 μM).

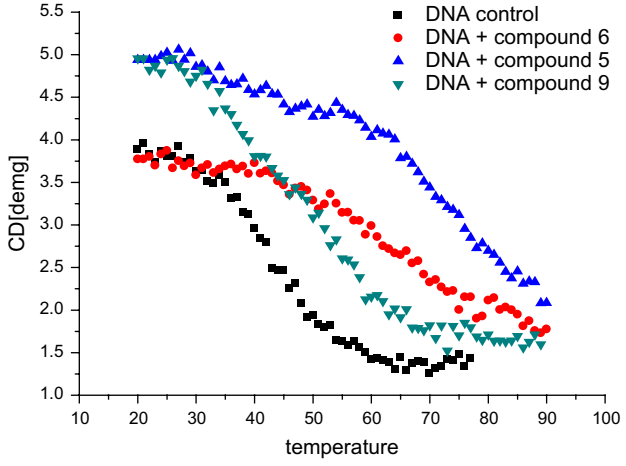


Figure 5. CD curves of G4-A in Na^+ buffer.

Table 2A. ΔT_m values of different compounds when $r = 2$ (r = [phthalocyanines]/[G4-A])

Compound	**1**	**2**	**3**	**4**	**5**
G4-A ΔT_m [°C]	6.0	No*	17.0	32.0	40.0
Compound	**6**	**7**	**8**	**9**	**10**
G4-A ΔT_m [°C]	21.0	30.0	29.0	4.0	6.0

G4-quadruplex; No* means that we cannot get the ΔT_m value.

Table 2B. ΔT_m values of different compounds when $r = 2$ (r = [phthalocyanines]/[DNA])

Compound	**3**	**4**	**5**	**8**	**9**
dsDNA ΔT_m [°C]	13.8	4.8	5.2	6.4	1.5

According to our experiment results, the T_m value was increased by 4.0–40 °C in the presence of different phthalocyanines when $r = 2$ (r = [phthalocyanines]/[DNA]) (shown in Table 2A). It is clear that compounds **3**, **4**, **5**, **6**, **7**, **8** could increase the melting temperature of the G-quadruplex ($\Delta T_m > 17.0$ °C), which indicated a high affinity of some phthalocyanines to the human telomeric sequence resulting in increased stability of the telomeric G-quadruplex. These DNA melting data were a strong evidence of G-quadruplex stabilization.

We know that the selectivity of G-quadruplex recognition is an important study for the G-quadruplex binding.[31] In order to demonstrate the selectivity of G-quadruplex by phthalocyanines, we used double stranded DNA as control to determine their ΔT_m by UV. UV-thermal transitions of dsDNA were examined at 260 nm, which is a typical index for the conformational change of dsDNA.[32] Our results showed that compounds **1**, **2**, **6**, **7**, **10** could not increase the T_m value of dsDNA ($\Delta T_m = 0$, data not shown), which indicated that these compounds could not stabilize the dsDNA. The ΔT_m values of other compounds to dsDNA are shown in Table 2B. ΔT_m values of most G-quadruplex DNA–phthalocyanine complexes were much higher than those of dsDNA–phthalocyanine complexes. The results here demonstrated that synthesized phthalocyanines could selectively stabilize G-quadruplex.

3. Conclusions

Several cationic phthalocyanine derivatives (**1**–**10**) were designed and synthesized, and their interactions with the telomeric G-quadruplex have been studied. Introducing oxygen, sulfur group to the periphery of phthalocyanines and different metals to the cores of the phthalocyanines, we found that the water-soluble cationic phthalocyanine derivatives stabilize G-quadruplex and inhibit telomere elongation. The modified telomerase repeat amplification protocol (TRAP) assay demonstrated that the inhibitory effect of some compounds on telomerase was at nanomole. Polymerase stop assay indicated that these compounds could stabilize the G-quadruplex. Phthalocyanine derivatives with different structures had abilities to stabilize the G-quadruplexes as determined by PCR stop experiment. CD melting studies show that most of compounds could selectively stabilize G-quadruplex. Further medicinal application will be investigated in the future.

4. Experiment

4.1. Chemistry

4,5-Dichlorophthalonitrile was purchased from TCI. 2-(Diethylamino)ethanethiol hydrochloride, 2-(dimethylamino)ethanethiol hydrochloride, 1,3-bis(dimethylamino)-2-propanol, 2-(dimethy-lamino)ethanol hydrochloride was purchased from Aldrich. K_2CO_3, DBU, methyl iodide, *n*-pentanol, acetic acid dichloromethane, methanol, ethanol, acetone, and DMF were from Shanghai Medical.Com, Ltd. DMF were stirred with calcium hydride for 2 h and then carefully distilled to avoid moisture and kept with 4 Å molecular sieves before use.

NMR spectra were recorded on a Varian Mercury-VX300 spectrometer at 300 MHz. MS were recorded on a Brucker Daltonics APE XII 47e and VG-707VHF mass spectrometer. UV/Vis spectra were recorded on a Scinco S-3100 Spectrophotometer. TG was performed in Setaram Setsys 16 TG-DPA/DSC.

4.2. General procedure for compounds 11–16

Compounds **11**–**16** were synthesized referring to the literature.[9–14] A mixture of different phthalonitriles, corresponding alcohol, and K_2CO_3 in DMF was stirred at 35 °C for 48 h. The reaction mixture was poured into ice-water and then was extracted with $CHCl_3$. The organic extracts were dried over anhydrous Na_2SO_4 and concentrated under vacuum to give yellow oil. After the crude product was purified by silica gel chromatography with an eluent $CHCl_3/CH_3OH$, 20:1, and compounds **11**–**16** were obtained.

4.2.1. 4-Diethylaminoethylsulfanyl 1,2-dicyano-benzene (11).[9] A mixture of 4-nitrophthalonitrile (358 mg, 2.07 mmol) and 1,2-(diethylamino)-ethanethiol hydrochloride (253 mg, 1.5 mmol) with K_2CO_3 (2.1 g, 16 mmol) in anhydrous DMF was stirred at 35 °C for 96 h. Then the reaction was treated according to 5.1.1. Yield: 310 mg (80%). ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 7.56$ (d, $J = 9$ Hz, 1H), 7.46 (dd, $J_1 = 1.8$ Hz, $J_2 = 8.1$ Hz, 1H), 7.20 (s, 1H), 3.05 (t, $J = 7.2$ Hz, 2H), 2.70 (dd, $J_1 = 7.2$ Hz, $J_2 = 6.6$ Hz, 2H), 2.52 (m, 4H), 0.97 (dd, $J_1 = 7.5$ Hz, $J_2 = 6.6$ Hz, 4H).

4.2.2. 4-Dimethylaminoethylsulfanyl 1,2-dicyano benzene (12)[10]. A mixture of 4-nitrophthalonitrile (400 mg, 2.3 mmol) and 1,2-(dimethylamino)-ethanethiol hydrochloride (280 mg, 2 mmol) with K_2CO_3 (2.5 g, 19 mmol) in anhydrous DMF was stirred at 35 °C for 96 h. Then the reaction was treated according to 5.1.1. Yield: 360 mg (78%). ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 7.65$ (d, $J = 8.1$ Hz, 1H), 7.58 (d, $J = 1.5$ Hz, 1H), 7.51 (dd, $J_1 = 2.1$ Hz, $J_2 = 8.7$ Hz, 1H),

3.32 (t, J = 6.6 Hz, 2H), 2.65 (dd, J_1 = 7.2 Hz, J_2 = 6.6 Hz, 2H), 2.30 (s, 6H).

4.2.3. 4-[1,3-Bis-(dimethylamino)2-propyloxy]1,2dicyano-benzene (13)[11]. A mixture of 4-nitrophthalonitrile (358 mg, 2.07 mmol) and 1,3-(dimethylamino)-2-propanol (278 mg, 1.5 mmol) with K_2CO_3 (2.3 mg, 17 mmol) in anhydrous DMF was stirred at 35 °C for 96 h. Then the reaction was treated according to 5.1.1. Yield: 306 mg (75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.64 (d, J = 8.7 Hz, 1H), 7.34 (d, J = 3 Hz, 1H), 7.26 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 1H), 4.50 (dd, J_1 = 5.7 Hz, J_2 = 5.1 Hz, 1H), 2.51 (d, J = 5.1 Hz, 4H), 2.21 (s, 12H).

4.2.4. 4-Dimethylaminoethyloxygenyl 1,2-dicyano benzene (14)[12]. A mixture of 4-nitrophthalonitrile (500 mg, 2.89 mmol) and 1,2-(dimethylamino)-ethanol (356 mg, 4 mmol) with K_2CO_3 (4 g, 31 mmol) in anhydrous DMF was stirred at 35 °C for 96 h. Then the reaction was treated according to 5.1.1. Yield: 565 mg (91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.70 (d, J = 9 Hz, 1H), 7.28 (d, J = 2.7 Hz, 1H), 7.23 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 1H), 4.16 (t, J = 5.4 Hz, 2H), 2.80 (t, J = 5.1 Hz, 2H), 2.36 (s, 6H).

4.2.5. 1,2-Bis-(diethylaminoethylsulfanyl)-4,5-dicyano benzene (15)[13]. A mixture of 4,5-dichlorophthalonitrile (390 mg, 2 mmol) and 1,2-(dimethylamino)-ethanethiol hydrochloride (588 mg, 4.2 mmol) with K_2CO_3 (3.5 g, 27 mmol) in anhydrous DMF was stirred at 35 °C for 96 h. Then the reaction was treated according to 5.1.1. Yield: 474 mg (71%) ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.52 (s, 2H), 3.10 (t, J = 7.5 Hz, 4 H), 2.78 (dd, J_1 = 7.2 Hz, J_2 = 6.6 Hz, 4H), 2.59 (m, 8H), 1.03 (m, 12H).

4.2.6. 1,2-Bis-(dimethylaminoethylsulfanyl)-4,5-dicyano benzene (16)[14]. A mixture of 4,5-dichlorophthalonitrile (350 mg, 1.79 mmol) and 1,2-(diethylamino) ethanethiol hydrochloride (672 mg, 4 mmol) with K_2CO_3 (3.2 g, 25 mmol) in anhydrous DMF was stirred at 35 °C for 96 h. Then the reaction was treated according to 5.1.1. Yield: 509 mg (73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.48 (s, 4H), 3.12 (dd, J_1 = 6.6 Hz, J_2 = 7.2 Hz, 4H), 2.66 (dd, J_1 = 6.6 Hz, J_2 = 7.2 Hz, 4H), 2.30 (s, 12H).

4.3. General procedure for compounds 17, 18, 22, 25, 26

A solution of substituted phthalonitriles and a few drops of 1,8-diazabicyclo (5.4.0) undec-7-ene (DBU) (1.00 mmol) in n-pentanol was heated to reflux for 25 h. in the presence of Zn $(OAc)_2$. Then the mixture was treated with aqueous acetic acid, washing the aqueous solution with several solvents (Et_2O, AcOEt, CH_2Cl_2, $CHCl_3$). After basification with K_2CO_3 and extraction of the neutral compound with $CHCl_3$, the volatiles were removed under reduced pressure. Purification of the crude products was performed by silica gel chromatography (eluent $CHCl_3$: MeOH, 3: 2), and the compounds **17**, **18**, **22**, **25**, **26** were obtained.

4.3.1. Tetrakis(2-diethylaminoethylsulfanyl)phthalocyanine zinc (17). A solution of 4-diethylaminoethylsulfanyl 1,2-dicyanobenzene(257 mg, 1 mmol) and 1,8-diazabicyclo (5.4.0) undec-7-ene (DBU) (1.00 mmol) in 8 mL 1-pentanol was heated to reflux for 25 h in the presence of Zn $(OAc)_2{\cdot}4H_2O$ (64 mg,0.25 mmol). Then the reaction was treated according to 5.1.2. Yield:123 mg (45%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.72 (br, 8H), 7.93 (br, 4H), 3.57 (br, 8H), 3.05 (br, 8H), 2.76 (br, 16H), 1.17 (br, 24H). ^{13}C NMR δ = 152.20, 138.81, 135.13, 128.51, 122.71, 120.59, 52.63, 47.31, 31.51, 12.88. HRMS (ESI) MH^+: 1101.3952 (calcd mass for $C_{56}H_{68}N_{12}S_4Zn$ MH^+ 1101.3937). UV/Vis ($CHCl_3$): λ_{max} (log ε) = 362 (3.9), 656 (3.8), 690 (3.7), 755 nm (3.7).

4.3.2. Tetrakis(2-dimethylaminoethylsulfanyl)phthalo-cyanine zinc (18). A solution of 4-dimethylaminomethylsulfanyl 1,2-dicyanobenzene (241 mg, 1 mmol) and 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) (1.00 mmol) in 8 mL n-pentanol was heated to reflux for 25 h. in the presence of $Zn(OAc)_2{\cdot}4H_2O$ (64 mg, 0.25 mmol).Then the mixture was treated according to 5.1.2. Yield: 74 mg (30%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.94 (br, 4H), 8.67 (br, 4H), 7.61 (br, 4H), 5.05 (br, 8H), 2.89 (m, 8H), 2.49 (br, 24H). ^{13}C NMR δ = 156.51, 152.02, 139.10, 135.66, 128.79, 122.94, 120.79, 58.55, 45.50, 30.93. HRMS (ESI) MH^+: 989.2681 (calcd mass for $C_{48}H_{52}N_{12}S_4Zn$ MH^+ 989.2685). UV/Vis ($CHCl_3$): λ_{max} (log ε) = 364 (4.0), 631 (3.9), 695 nm (4.0).

4.3.3. Tetrakis(2-dimethylaminoethylethanol)phthalo-cyanine zinc (22)[12]. A solution of 4-dimethylaminoethyloxygenyl 1,2-dicyanobenzene (215.11 mg, 1 mmol) and 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) (1.00 mmol) in 8 mL 1-pentanol was heated to reflux for 25 h in the presence of $Zn(OAc)_2{\cdot}4H_2O$ (64 mg, 0.25 mmol). Then the reaction was treated according to 5.1.2. Yield: 32 mg (14%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.29 (br, 4H), 7.67 (br, 8H), 4.57 (br, 8H), 2.89 (br, 8H), 2.38 (br, 24H). HRMS (ESI) MH^+: 925.3598 (calcd mass for $C_{48}H_{52}N_{12}O_4Zn$ MH^+ 925.3599). UV/Vis ($CHCl_3$): λ_{max} (log ε) = 283 (3.6), 359 (3.9), 616 (3.5), 679 nm (3.9).

4.3.4. Octakis (2-dimethylaminoethylsulfanyl)phthalocyanine zinc (25)[20]. A solution of 1,2-Bis-(dimethyl aminoethyl sulfanyl)-4,5-dicyanobenzene (334.13 mg, 1 mmol) and 1,8-diazabicyclo (5.4.0) undec-7-ene (DBU) (1.00 mmol) in 8 mL 1-pentanol was heated to reflux for 25 h. Then the reaction was treated according to 5.1.2. Yield: 48 mg (14%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.61 (br, 8H), 3.51 (br, 16H), 2.99 (br, 16H), 2.71 (br, 32H), 1.10 (t, J_1 = 6.3 Hz, J_2 = 6.9 Hz, 48H). HRMS (ESI+) MH^+: 1626.7056 (calcd mass for $C_{80}H_{120}N_{16}S_4Zn$ MH^+ 1626.7012). UV/Vis ($CHCl_3$): λ_{max} (log ε) = 364 (3.9), 676 (3.8), 780 nm (3.7).

4.3.5. Octakis(2-diethylaminoethylsulfanyl)phthalocyanine zinc (26).[16] A solution of 1,2-bis-(diethylamino ethyl sulfanyl)-4,5-dicyanobenzene (390.19 mg, 1 mmol) and 1,8-diazabicyclo (5.4.0) undec-7-ene (DBU) (1.00 mmol) in 8 mL n-pentanol was heated to reflux

for 25 h. Then the reaction was treated according to 5.1.2. Yield: 70 mg (17%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.19 (s, 8H), 3.72 (br, 16, H), 3.15 (br s, 16H), 2.65 (br, 48H). HRMS (ESI) MH$^+$: 1401.5579 (calcd mass for $C_{64}H_{88}N_{16}S_8Zn$ MH$^+$ 1401.5513). UV/Vis ($CHCl_3$): λ_{max} (log ε) = 328 (3.8), 365 (4.0), 676 (3.8), 715 nm (3.9).

4.4. General procedure for compounds 19, 23

A solution of substituted phthalonitriles, a few drops of 1,8-diazabicyclo (5.4.0)undec-7-ene (DBU) (1.00 mmol) and $Ni(OAc)_2{\cdot}2H_2O$ (62.21 mg, 0.25 mmol) in *n*-pentanol were heated to reflux for 25 h. Then the mixture was treated with aqueous acetic acid, washing the aqueous solution with several solvents (Et_2O, AcOEt, CH_2Cl_2, $CHCl_3$). After basification with K_2CO_3, extraction of the neutral compound with $CHCl_3$, and the solvents were removed under reduced pressure. Purification of the crude product was finished by silica gel chromatography (eluent $CHCl_3$/MeOH, 3:2), and then the compounds **19**, **23** were obtained.

4.4.1. Tetrakis(4-[1,3-bis-(dimethylamino)2-propyloxy])phthalocyanine nickel (19). 2-[1,3-bis-(dimethylamino)2-propyloxy]1,2-dicyano benzene (272 mg, 1 mmol), $Ni(OAc)_2{\cdot}2H_2O$ (62.21 mg,0.25 mmol) and 1,8-diazabicyclo (5.4.0) undec-7-ene (DBU) (1.00 mmol) in 8 mL *n*-pentanol was heated to reflux for 25 h. Then the mixture was treated with crude products according to 5.1.3. Yield: 34 mg (12%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.94 (m, 4H), 8.68 (m, 4H), 7.62 (m, 4H), 5.07 (m, 4H), 2.90 (br, 16H), 2.50 (br, 48H). ^{13}C NMR δ = 159.81, 144.11, 138.47, 130.20, 123.20, 199.22, 106.83, 76.55, 61.54, 47.06. HRMS (ESI+) MH$^+$: 1147.5968 (calcd mass for $C_{60}H_{80}N_{12}O_4Ni$ MH$^+$ 1147.5975). UV/Vis ($CHCl_3$): λ_{max} (log ε) = 300 (3.9), 383 (3.5), 609 (3.6), 673 nm (3.9).

4.4.2. Tetrakis(2-dimethylaminoethylethanol)phthalocyanine nickel (23). A solution of 4-dimethylaminoethyloxygenyl 1,2-dicyanobenzene (215.11 mg, 1 mmol) and 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) (1.00 mmol) in 8 mL 1-pentanol was heated to reflux for 25 h in the presence of $Ni(OAc)_2{\cdot}2H_2O$ (62.21 mg, 0.25 mmol). Then the mixture was treated according to 5.1.3. Yield: 18 mg (7.8 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.51 (m, 4H), 6.80 (m, 8H), 4.04 (br, 8H), 2.91 (br, 8H), 2.53 (br, 24H). ^{13}C NMR δ = 159.16, 141.15, 136.48, 128.51, 121.55, 117.39, 102.26, 65.84, 58.57, 46.34. HRMS (ESI) MH$^+$: 919.3531 (calcd mass for $C_{48}H_{54}N_{12}O_4Ni$ MH$^+$: 919.3588). UV/Vis ($CHCl_3$): λ_{max} (log ε) = 280 (3.8), 365 (3.4), 672 nm (3.8).

4.5. General procedure for compounds 20, 21, 24

A solution of substituted phthalonitrile and a few drops of 1,8-diazabicyclo (5.4.0) undec-7-ene (DBU) (1.00 mmol) in *n*-pentanol was heated to reflux for 36 h. Then the mixture was treated with aqueous acetic acid, washing the aqueous solution with several solvents (Et_2O, AcOEt, CH_2Cl_2, $CHCl_3$). After basification with K_2CO_3 and extraction of the neutral compound with $CHCl_3$, and the volatiles were removed under reduced pressure. Purification of the crude product was performed by silica gel chromatography (eluent $CHCl_3$/MeOH, 3:2), and the compounds **20**, **21**, **24** were obtained.

4.5.1. Tetrakis(2-diethylaminoethylsulfanyl)phthalocyanine (20). A solution of 4-diethylaminoethylsulfanyl 1,2-dicyanobenzene (257 mg, 1 mmol) and 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) (1.00 mmol) in 8 mL of *n*-pentanol was heated to reflux for 25 h. Reaction was treated according to 5.1.4. Yield: 12 mg (4.7%) ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.01 (m, 8H), 7.48 (m, 4H), 3.41 (m, 8H), 3.07 (br, 8H), 2.84 (m, 16H), 1.27 (m, 48H). ^{13}C NMR δ = 139.79, 135.39, 131.59, 127.61, 121.84, 119.78, 118.84, 52.26, 47.50, 30.95, 12.48. HRMS (ESI) MH$^+$: 1039.4816 (calcd mass for $C_{56}H_{68}N_{12}S_4$ MH$^+$ 1139.4802). UV/Vis ($CHCl_3$): λ_{max} (log ε) = 302 (4.0), 340 (4.1), 680 (4.0), 712 nm (4.0).

4.5.2. Tetrakis(2-dimethylaminoethylethanol)phthalo cyanine (21).[10] A solution of 4-dimethylaminoethyloxygenyl 1,2-dicyanobenzene (215.11 mg, 1 mmol) and 1,8-diazabicyclo (5.4.0) undec-7-ene (DBU) (1.00 mmol) in 8 mL 1-pentanol was heated to reflux for 25 h. Then the reaction was treated according to 5.1.4. Yield: 25 mg (12%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.66 (m, 4H), 6.91 (m, 8H), 4.10 (br, 8H), 3.07 (br, 8H), 2.55 (br, 24H). HRMS (ESI) MH$^+$: 863.4594 (calcd mass for $C_{48}H_{54}N_{12}O_4$ MH$^+$ 863.4591). UV/Vis ($CHCl_3$): λ_{max} (log ε) = 288 (3.5), 342 (3.7), 667 (3.8), 704 nm (3.8).

4.5.3. Tetrakis(2-dimethylaminoethylsulfanyl)phthalo cyanine (24). A solution of 4-dimethylaminomethylsulfanyl 1,2-dicyanobenzene (231 mg, 1 mmol) and 1,8-diazabicyclo (5.4.0) undec-7-ene (DBU) (1.00 mmol) in 8 mL of *n*-pentanol was heated to reflux for 25 h. Then the reaction was treated according to 5.1.4. Yield: 38 mg (16%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.66 (m, 8H), 7.26 (m, 4H), 3.30 (m, 8H), 2.90 (m, 8H), 2.51 (m, 24H). ^{13}C NMR δ = 146.50, 139.28, 134.95, 131.33, 127.53, 121.44, 119.10. HRMS (ESI) MH$^+$: 927.3432 (calcd mass for $C_{48}H_{56}N_{12}S_4$ MH$^+$ 927.3477). UV/Vis ($CHCl_3$): λ_{max} (log ε) = 299 (3.8), 342 (3.9), 421 (3.5), 680 (3.9), 714 nm (3.9).

4.6. General procedure for compounds 1–10

Compounds 17–**26** were dissolved in $CHCl_3$, and a large excess of methyl iodide was added to this solution. The mixture was refluxed for 4 h. After cooling to room temperature, the green precipitate was filtered off and washed with $CHCl_3$. Then the product was dissolved by the acetonitrile-methyl alcohol and dried and compounds were obtained **1**–**10**.

4.6.1. Tetrakis (*N*,*N*,*N*-diethyl-methyl-2-aminol-ethylsulfanyl)phthalocyaninato zinc tetraiodine (1). Compound **17** (30 mg, 0.027 mmol) was dissolved in $CHCl_3$ (5 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was refluxed for 4 h. Then the reaction was treated according to 5.1.5.Yield: 35 mg (95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.46 (br, 8H), 8.37 (br, 4H), 3.93 (br, 8 H), 3.71(br, 8H), 3.52 (br, 12H), 3.13 (br, 16H), 1.31 (br, 24H). ^{13}C NMR

δ = 151.58, 133.45, 138.77, 135.04, 128.44, 122.76, 120.32, 63.46, 58.85, 45.81, 31.31. UV/Vis (H_2O): λ_{max} ($\log\varepsilon$) = 353 (3.78), nm 656 (3.9). elemental analysis calcd (%) for $C_{60}H_{80}N_{12}S_4I_4Zn{\cdot}6H_2O$: C, 40.05; H, 5.18; N, 9.46; Found: C, 40.01; H, 4.91; N, 9.38. TG weight loss was found at 210 °C, $6H_2O$.

4.6.2. Tetrakis(2-trimethylaminolethylsulfanyl)phthalo cyaninato zinc tetraiodine (2). Compound **18** (25 mg, 0.027 mmol) was dissolved in $CHCl_3$ (5 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was refluxed for 4 h. Then the reaction was treated according to 5.1.5. Yield: 28 mg (88%). (Found: C, ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.39 (br, 8H), 8.29 (br, 4H), 3.91 (br, 8H), 3.80 (br, 8H), 3.29 (br, 36H). ^{13}C NMR δ = 153.09, 139.52, 137.04, 136.51, 130.42, 123.77, 121.51. UV/Vis (H_2O): λ_{max} ($\log\varepsilon$) = 344 (3.8), 642 nm (3.8). Elemental analysis calcd (%) for $C_{52}H_{64}N_{12}S_4I_4Zn{\cdot}6H_2O$: C, 37.67; H; 4.69; N, 9.88. Found: C, 37.50; H, 4.57; N, 10.1. TG weight loss was found at 210 °C, $6H_2O$.

4.6.3. 4-[1,3-Bis-(trimethylamino)2-propyloxy]phthalo-cyaninato zinc octaiodine (3). Compound **19** (25 mg, 0.021 mmol) was dissolved in $CHCl_3$ (10 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was refluxed for 4 h. Then the reaction was treated according to 5.1.5. Yield: 47 mg (96%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.37 (br, 8H), 8.20 (br, 4H), 6.30 (br, 4 H), 4.19 (br, 16H), 3.43 (br, 72H). ^{13}C NMR δ = 161.69, 150.99, 144.09, 137.33, 129.83, 126.84, 115.44, 74.51, 72.18, 59.514. UV/Vis (H_2O): λ_{max} ($\log\varepsilon$) = 168 (3.9), 667 nm (4.2). elemental analysis calcd (%) for $C_{60}H_{104}N_{16}O_4I_8Ni{\cdot}4H_2O$: C, 34.66; H, 4.79; N, 9.53. Found: C, 34.74; H, 4.79; N, 9.55. TG weight loss was found at 155 °C, $4H_2O$.

4.6.4. Tetrakis(*N*,*N*,*N*-diethyl-methyl-2-aminolethyl sulfanyl)phthalocyaninato tetraiodine (4). Compound **20** (18 mg, 0.021 mmol) was dissolved in $CHCl_3$ (5 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was refluxed for 4 h. Then the reaction was treated according to 5.1.5. Yield: 28 mg (93%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.34 (br, 8H), 8.40 (br, 4H), 3.98 (br, 8 H), 3.75 (br, 8H), 3.57 (br, 12H), 3.18 (br, 16H), 1.34 (br, 24H). ^{13}C NMR δ = 148.09, 138.47, 135.82, 133.18, 130.78, 123.47, 121.54, 59.17, 56.67, 47.67, 26.58, 8.61. UV/Vis (H_2O): λ_{max} ($\log\varepsilon$) = 331 (3.8), 638 nm (3.8). Elemental analysis calcd (%) for $C_{60}H_{82}N_{12}S_4I_4{\cdot}5H_2O$: C, 42.41; H, 5.42; N, 9.88. Found: C, 42.14; H, 5.11; N, 9.78. TG weight loss was found at 190 °C, $5H_2O$.

4.6.5. Tetrakis(2-trimethylaminoethylethanol)phthalocyaninato tetraiodine (5). Compound **21** (15 mg, 0.016 mmol) was dissolved in $CHCl_3$ (5 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was refluxed for 4 h. Then the reaction was treated according to 5.1.5. Yield: 22 mg (85%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.36 (br, 4H), 8.98 (br, 4H), 7.97 (br, 4 H), 5.11(br, 8H), 4.15 (br, 8H), 3.42 (br, 36H). ^{13}C NMR δ = 160.37, 148.33, 137.85, 129.71, 124.47, 119.69, 107.05, 65.15, 63.78, 54.26. UV/Vis (H_2O): λ_{max} ($\log\varepsilon$) = 328 (3.8), 632 nm (3.7). Elemental analysis calcd (%) for $C_{52}H_{66}N_{12}O_4I_4{\cdot}2H_2O$: C, 42.60; H, 4.64; N, 11.46. Found: C, 42.53; H, 4.81; N, 11.61. TG weight loss was found at 160 °C, $2H_2O$.

4.6.6. Tetrakis(2-trimethylaminoethylethanol)phthalo cyaninato zinc tetraiodine (6).[12] Compound **22** (30 mg, 0.032 mmol) was dissolved in $CHCl_3$ (5 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was refluxed for 4 h. Then the reaction was treated according to 5.1.5. Yield: 45 mg (93%) ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.33 (br, 4H), 8.97 (br, 4H), 7.88 (br, 4 H), 5.09 (br, 8H), 4.09 (br, 8H), 3.38 (br, 36H). ^{13}C NMR δ = 160.16, 152.68, 140.57, 132.27, 124.39, 119.18, 107.11, 65.21, 63.67, 54.19. UV/Vis (H_2O): λ_{max} ($\log\varepsilon$) = 337 (3.9), 633 nm (3.9).

4.6.7. Tetrakis(2-trimethylaminoethylethanol)phthalo cyaninato nickel tetraiodine (7). Compound **23** (20 mg, 0.0216 mmol) was dissolved in $CHCl_3$ (5 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was refluxed for 4 h. Then the reaction was treated according to 5.1.5. Yield: 30 mg (94%). ^{1}H NMR (300 MHz, DMSO-d_6) ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.89 (br, 8H), 7.77 (br, 4H), 5.04 (br, 8H), 4.24 (br, 8 H), 3.48 (br, 36H). ^{13}C NMR δ = 160.13, 148.28, 137.56, 129.46, 124.21, 119.57, 106.79, 65.15, 63.76, 54.35. UV/Vis (H_2O): λ_{max} ($\log\varepsilon$) = 276 (3.8), 622 nm (3.7). Elemental analysis calcd (%) for $C_{52}H_{64}N_{12}O_4I_4Ni{\cdot}5H_2O$: C, 39.55; H, 4.69; N, 10.65. Found: C, 39.60; H, 4.57; N, 10.70. TG 6.2; TG weight loss was found at 200 °C, $5H_2O$.

4.6.8. Tetrakis(2-trimethylaminolethylsulfanyl)phthalo cyaninato tetraiodine (8). Compound **24** (30 mg, 0.027 mmol) was dissolved in $CHCl_3$ (5 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was refluxed for 4 h. Then the reaction was treated according to 5.1.5. Yield: 31 mg (95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.36 (br, 4H), 8.98 (br, 4H), 7.97 (br, 4 H), 5.11 (br, 8H), 4.15 (br, 8H), 3.42 (br, 36H). ^{13}C NMR δ = 152.56, 143.22, 140.51, 137.76, 135.12, 128.25, 126.86, 69.34, 58.19, 31.72. UV/Vis (H_2O): λ_{max} ($\log\varepsilon$) = 331 (3.7), 637 nm (3.7). Elemental analysis calcd (%) for $C_{52}H_{66}N_{12}S_4I_4{\cdot}2H_2O$: C, 40.75; H, 4.57; N, 10.98. Found: C, 40.59; H, 4.71; N, 10.77. TG 6.2; TG weight loss was found at 190 °C, $2H_2O$.

4.6.9. Octakis(2-trimethylaminoethylsulfanyl)phtha-locyaninato zinc octaiodine (9).[14] Compound **25** (33 mg, 0.025 mmol) was dissolved in $CHCl_3$ (5 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was refluxed for 4 h. Then the reaction was treated according to 5.1.5. Yield: 56 mg (91%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.62 (s, 8H), 4.05 (br, 16H), 3.80 (br, 16 H), 3.61 (s, 24H), 3.25 (br, 32H), 1.35 (s, 48H). ^{13}C NMR δ = 162.98, 154.77, 153.55, 137.59, 58.75, 56.70, 47.59, 26.59, 8.57. UV/Vis (H_2O): λ_{max} ($\log\varepsilon$) = 354 (3.9), 657 nm (4.0).

4.6.10. Octakis (*N*,*N*,*N*-diethyl-methyl-2-aminoethyl sulfanyl)phthalocyanine zinc octaiodine (10).[16] Compound

26 (36 mg, 0.023 mmol) was dissolved in $CHCl_3$ (5 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was refluxed for 4 h. Then the reaction was treated according to 5.1.5. Yield: 55 mg (89%). ^1H NMR (300 MHz, DMSO-d_6) $\delta = 9.51$ (s, 8H), 4.04 (br, 16H), 3.93 (br, 16H), 3.36 (s,72H). ^{13}C NMR $\delta = 1153.60$, 137.65, 137.49, 124.10, 64.10, 64.41, 53.53, 27.33. UV/Vis (H_2O): λ_{max} ($\log\varepsilon$) = 365 (4.0), 676 (3.8), 715 nm (3.9).

4.7. Biology

4.7.1. Oligonucleotides. All oligomers used in this study were purchased from Invitrogen (China). G4A DNA_4: 5′-CATGG TGGTTT GGGTTA GGGTTA GGGTTA GGGTTA CCAC-3′ dsDNA 5′-GCATTGGTAAC TGTCAGACC-3′ and its complementary strand 5′-G GTCTGACAG-TTACCAATGC-3′ were used for UV melting. The template sequence HT4 (5′-TCCAA CTATG TATAC TTAGGG TTAGGG TTAGGG TTAGGG ACATA TCGAT GAAAT TGCTA TAG TG AGTCG TATTA-3′) and 5′ Tamra labeled P18 (5′-TAATA CGACT CACTATAG-3′) (TaKaRa Biotechnology) were used for DNA polymerase stop assay. **Seq1**: 5′-GGGTTAGGGTTAGGGTTAGGG-3′, **Seq2**: 5′-TCTCGTCTTCCCTAA-3′, **Seq3**: 5′-GGGTTAG AATTAGGGTTAGGG-3′, Taq DNA polymerase were purchased from TOYOBO (Japan).

4.7.2. TRAP assay. Assay was performed according to the reported method.[33,34] Every reaction was performed in 25 μL reaction mixture including 20 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.05% Tween 20, 1 mM EGTA, 50 μM each dNTPs, 0.01 μg TS primer (5′-AATCCGTCGA GCAGAGTT-3′), 0.01 μg ACX primer (5′-GCG CGGCTTACCCTTACCCTT AC CCTAACC-3′), 0.01 μg NT primer(5′-ATCGC TTCT CGGCCTTTT-3′), 0.01 μmol internal control TSNT primer (5′-AATCCGTCGAGCAGAGTTAAAAGGC CGAGAAGCGAT-3′), 2 U Taq polymerase (Toyabo, Japan), 500 cells of HeLa cell extract, and appreciated concentrations of compounds. After drug treatment in 10 min, telomerase extension reaction was performed at 30 °C for 30 min. Then the reaction was stopped by heating to 94 °C for 5 min. PCR amplification was performed with 33 cycles at 94 °C for 30 s and 59 °C for 30 s and PCR products were analyzed by electrophoresis on 12% polyacrylamide nondenaturing gel and stained with EB. Photograph was imaged by Chemilmager 5500 and quantitation of the bands in each ladder was performed by comparing to the ITAS. After calculating the telomerase activity relative to the control, the IC_{50} (0.25 μM) was determined by graph sigmoidal fit.

4.7.3. DNA polymerase stop assay. The procedure was described by Hurley and coworker.[4,35] Primer (P18, 180 nM) labeled with Tamra was mixed with template DNA (HT4) (180 nM) in a Tris–HCl buffer (10 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$) containing 50 mM K^+ and denatured by heating at 90 °C for 5 min. After cooling down to room temperature, phthalocyanines were added at various concentrations, and the mixture was incubated with dNTPs (final concentration 200 μM), Taq DNA polymerase (1.5 U/reaction; TOYOBO) at 55 °C for 30 min. The reactions were stopped by adding an equal volume of stop buffer (95% formamide, 10 mM NaOH). The products were separated on a 20% denaturing polyacrylamide gel for electrophoresis and autoradiographed on a Typhoon phosphorimager (Amersham Biosciences, AB, Uppsala, Sweden).

4.7.4. PCR stop. Assay was performed according to the reported method.[26] Every reaction was carried out in 12.5 μL of reaction mixture including 20 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 50 μM dNTP, the phthalocyanine derivatives at various concentrations, 10 μM 5′-GGGTTAGGGTTAGGGTTAG GG-3′ (or 10 μM 5′-GGGTTAGAAGGTTAGGGT TAGGG-3′), and 10 μM 5′-TCTCGTCTTCCCTAA-3′. Reaction mixtures were incubated in a thermocycle with the following cycling conditions: 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. PCR products were observed and analyzed by electrophoresis on 16% non-denaturing polyacrylamide gels in 1× BE and stained with EB.

4.7.5. CD and UV thermal melting studies. Solutions of 1.0 μM G quadruplex DNA were prepared in a Na^+ buffer (10 mM Tris–HCl, 1 mM EDTA, 100 mM NaCl, pH 7.4). The solution was first heated to 95 °C for 5 min and then cooled down to room temperature overnight. DsDNA and its complementary strand were annealed and allowed to form duplexes by heating the mixture solution at 95 °C for 5 min and then slowly cooling down to room temperature. Corresponding amounts of stock solutions of the compounds were added and G4 melting curves were obtained by monitoring the absorbance at 295 nm on a Jasco-810 spectropolarimeter (Jasco, Easton, MD) equipped with a digital circulating water bath. The temperature was controlled to ramp from 25 to 95 °C at about 0.5–1.5 °C/min and the influence on the absorbance change of each compound in the case of increasing temperature was determined. Melting profiles were analyzed by fitting them to a concerted two-state model while dsDNA melting curves were obtained by reading the absorbance at 260 nm on a Scinco S-3100 equipped with a digital circulating water bath.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.09.037.

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