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Synthesis and antiproliferative activity of 2,7-diamino l0-(3,5-dimethoxy)benzyl-9(10H)-acridone derivatives as potent telomeric G-quadruplex DNA ligands

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ABSTRACT

A novel series of l0-(3,5-dimethoxy)benzyl-9(10H)-acridone derivatives with terminal ammonium substituents at C2 and C7 positions on the acridone ring were successfully synthesized as antiproliferation agents. The biologic activity of the acridone compounds against leukemia CCRF-CEM cells demonstrated that some of the compounds displayed good antiproliferative activity, among which compound **6a** containing dimethylamine substituents at the terminal C2 and C7 positions exhibited the highest cytotoxicity with IC_{50} at 0.3 μM. In addition compound **6a** showed little toxicity against normal 293T cells proliferation with IC_{50} more than 100 μM. Further study indicated that compound **6a** had strong binding activity to human telomeric G-quadruplex DNA, as detected by mass spectrometry, CD spectroscopy, UV absorption, FRET and fluorescence quenching assays. Our data suggested that the activity of **6a** might be associated with its stabilization of G-quadruplex DNA, which can be developed as potent antitumor agent.

1. Introduction

Cancer has become one of the most serious diseases in the present society. One of the well-know hallmarks of cancer cells is their ability to sustain chronic proliferation [1,2]. Therefore great efforts have been made to identify new entities with good antiproliferative activity. As most of antitumor agents have various adverse effects, such as toxicity against normal cells, design and development of new compounds with good antiproliferative activity and low toxic effects on normal cells has attracted great attention [3–5].

Telomerase is a cellular reverse transcriptase which can add telomere repeats to telomere DNA to maintain chromosome integrity and stability. Most normal somatic cells lack telomerase activity, and after each cell division they would continually shorten their telomeres due to the end replication problem, until they enter replicative senescence. As 85–90% cancer cells display telomerase activity, which renders tumor cells with a capacity to continue cellular proliferative and to be immortal, telomerase can be regarded as an important target for cancer chemotherapy [6–9]. In addition, telomerase needs a linear, non-folded telomere DNA substrate to extend telomeres. If the telomere overhang DNA forms higher order structures, such as G-quadruplex, telomerase cannot recognize them and the activity of telomerase would be inhibited. Thus the design and synthesis of efficient compounds with the ability to stabilize G-quadruplex DNA is a potent strategy for anticancer chemotherapy [10–13].

Acridine and acridone derivatives show potent antitumor activity *in vitro*/*vivo*, some of which have been developed as good G-quadruplex stabilizers and antitumor agents, such as BRACO-19 and AS1410 [14–26]. Previously, we have reported that compounds based on planar tricyclic chromophore scaffolds exhibited antiproliferative activity *in vitro*, among which 10-(3,5-dimethoxy)benzyl-9(10H)-acridone with terminal amino substituents at C2 position on the acridone ring had strong binding activity to calf thymus DNA [27]. In order to further improve the

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anticancer activity and reduce the cytotoxicity to normal cells, two 10-(3,5-dimethoxy)benzyl-9(10H)-acridone with two terminal amino substituents at C2 and C7 positions on the acridone ring were developed as selective G-quardruplex stabilizers [28]. However, the two compounds displayed moderate antiproliferative activity. In continuation of our interest on the development of new acridones with potent antiproliferative activity, this study, we report the synthesis and biochemistry of new acridone derivatives with two terminal amino substituents at C2 and C7 positions to discover new antiproliferative entities.

2. Experimental

2.1. Materials and methods

Melting points (mp) were recorded on a SGW X-4 melting point apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR in DMSO-*d*6 solution with tetramethylsilane as the internal standard, respectively. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet. HRMS were recorded on a QSTAR XL spectrometer and Waters Q-Tof Premier spectrometer.

CCRF-CEM leukemia cells, human lung adenocarcinoma cells (A549), human breast adenocarcinoma cells (MCF7) and human embryonic kidney cells (293T), human leukemia cells (K562), and Human colon carcinoma cells (HCT-116) were purchased from the Cell Bank of Chinese Academy of Sciences.

Single-stranded oligonucleotides $(TTAGGG)_4$ were purchased from invitrogen (Guangdong, China), which were converted to the G-quadruplex according to our previous paper and references [28,29]. The concentration of quadruplex was determined spectroscopically.

2.2. Chemistry

2.2.1. Synthesis of 2,7-bis(3-chloropropionamido)-10-(3,5-dimethoxybenzyl)9,10-dihydro-acrid-inone (**5**)

A suspension of 4 (375 mg, 1 mmol), 3-chloropropanoyl chloride (382 mg, 3 mmol) and N-methyl morpholine (303 mg, 3 mmol) was refluxed in dry tetrahydrofuran until TLC indicated completion of reaction and then water was added with rapid stirring. Yellow solids were obtained after filtration. The solids were washed with ethanol to afford 5 (501 mg, 90%) as yellow solids, which were used without further purification.

2.2.2. General aminolysis procedure of compound (**6a**–**6g**)

To a stirred refluxing suspension of 5 (111 mg, 0.2 mmol) and KI (66 mg, 0.4 mmol) in EtOH (2 mL) was added to the corresponding secondary amines (5 mmol). The mixture was stirred at reflux until TLC indicated completion of reaction. After cooling to room temperature, water was added with rapid stirring under ice-water bath. Yellow solids were obtained after filtration. The products were purified by recrystallization from cold ethanol.

*2.2.2.1. 2,7-Bis[3-(dimethylamino)propionamido]-10-(3,5-dimethoxybenzyl)-9,10-di-hydroacridone (**6a**).* Yield 94 mg, 82%; m.p. 246–247 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.18 (s, 12H), 2.47 (t, 4H, ^3J = 7.2 Hz), 2.58 (t, 4H, ^3J = 7.2 Hz), 3.66 (s, 6H), 5.69 (s, 2H), 6.26 (m, 2H), 6.41 (m, 1H), 7.61 (d, 2H, ^3J = 9.6 Hz), 7.93 (dd, 2H, ^3J = 9.6 Hz, ^5J = 2.4 Hz), 8.61 (d, 2H, ^5J = 2.4 Hz), 10.31 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 35.0, 45.2, 49.1, 55.4, 98.6, 104.3, 115.5, 116.9, 121.5, 126.7, 133.6, 138.1, 139.2; HRMS calcd for $C_{32}H_{40}N_5O_5$[M + H]$^+$574.3029, found 574.3025.

*2.2.2.2. 2,7-Bis[3-(diethylamino)propionamido]-10-(3,5-dimethoxybenzyl)-9,10-di-hydroacridone (**6b**).* Yield 107 mg, 85%; m.p. 235–236 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.98 (t, 12H, ^3J = 7.2 Hz), 2.42–2.53 (m, 12H), 2.76 (t, 4H, ^3J = 7.2 Hz), 3.66 (s, 6H), 5.69 (s, 2H), 6.26 (m, 2H), 6.41 (m, 1H), 7.31 (m, 1H), 7.60 (d, 2H, ^3J = 9.6 Hz), 7.93 (dd, 2H, ^3J = 9.6 Hz, ^5J = 2.4 Hz), 8.59 (d, 2H, ^5J = 2.4 Hz), 10.31 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.1, 34.5, 46.4, 48.6, 55.4, 98.6, 104.3, 115.4, 116.9, 121.5, 126.7, 133.7, 138.1, 139.2, 161.2, 170.7, 176.3; HRMS calcd for $C_{36}H_{48}N_5O_5$[M + H]$^+$630.3655, found 630.3651.

*2.2.2.3. 2,7-Bis[3-(diethanolamino)propionamido]-10-(3,5-dimethoxybenzyl)-9,10-di-hydroacridone (**6c**).* Yield 76 mg, 55%; m.p. 205–206 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.46 (t, 4H, ^3J = 6.6 Hz), 2.59 (t, 8H, ^3J = 6.2 Hz), 2.85 (t, 4H, ^3J = 6.6 Hz), 3.47 (m, 8H), 3.66 (s, 6H), 4.40 (t, 4H, ^3J = 5.3 Hz), 5.69 (s, 2H), 6.26 (m, 2H), 6.41 (m, 1H), 7.31 (m, 1H), 7.60 (d, 2H, ^3J = 9.4 Hz), 7.92 (dd, 2H, ^3J = 9.4 Hz, ^5J = 2.5 Hz), 8.63 (d, 2H, ^5J = 2.5 Hz), 10.36 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 35.0, 49.4, 51.2, 55.6, 56.7, 59.7, 98.8, 104.6, 115.8, 117.0, 121.7, 127.1, 133.9, 138.4, 139.5, 161.4, 171.0, 176.5; HRMS calcd for $C_{36}H_{48}N_5O_9$[M + H]$^+$ 694.3452, found 694.3452.

*2.2.2.4. 2,7-Bis(3-pyrrolidinopropionamido)-10-(3,5-dimethoxybenzyl)-9,10-di-hydroacridone (**6d**).* Yield 100 mg, 80%; m.p. 245–246 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.69 (m, 8H), 2.48–2.52 (m, 12H), 2.74 (t, 4H, ^3J = 6.8 Hz), 3.66 (s, 6H), 5.69 (s, 2H), 6.26 (m, 2H), 6.41 (m, 1H), 7.61 (d, 2H, ^3J = 9.6 Hz), 7.92 (dd, 2H, ^3J = 9.6 Hz, ^5J = 2.4 Hz), 8.61 (d, 2H, ^5J = 2.4 Hz), 10.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 23.4, 36.4, 49.6, 51.8, 53.7, 55.4, 98.6, 104.3, 115.5, 116.9, 121.5, 126.7, 133.7, 138.1, 139.2, 161.2, 170.4, 176.3; HRMS calcd for $C_{36}H_{44}N_5O_5$[M + H]$^+$626.3342, found 626.3344.

*2.2.2.5. 2,7-Bis(3-piperidinopropionamido)-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridone (**6e**).* Yield 111 mg, 85%; m.p. 229–230 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.39 (m, 2H), 1.51 (m, 8H), 2.39 (m, 8H), 2.48 (t, 4H, ^3J = 6.8 Hz), 2.62 (t, 4H, ^3J = 6.8 Hz), 3.65 (s, 6H), 5.68 (s, 2H), 6.26 (m, 2H), 6.41 (m, 1H), 7.60 (d, 2H, ^3J = 9.2 Hz), 7.92 (d, 2H, ^3J = 9.2 Hz), 8.59 (brs, 1H), 10.33 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 24.3, 25.9, 34.3, 49.2, 53.9, 54.7, 55.4, 98.6, 104.3, 115.4, 116.9, 121.5, 126.7, 133.6, 138.2, 139.2, 161.2, 170.6, 176.3; HRMS calcd for $C_{38}H_{48}N_5O_5$[M + H]$^+$ 654.3655, found 654.3653.

*2.2.2.6. 2,7-Bis(3-morpholinopropionamido)-10-(3,5-dimethoxybenzyl)-9,10-di-hydro-acridone (**6f**).* Yield 113 mg, 86%; m.p. 265–266 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.42 (m, 8H), 2.51 (t, 4H, ^3J = 6.8 Hz), 2.65 (t, 4H, ^3J = 6.8 Hz), 3.58 (m, 8H), 3.66 (s, 6H), 5.69 (s, 2H), 6.26 (m, 2H), 6.41 (m, 1H), 7.61 (d, 2H, ^3J = 9.2 Hz), 7.93 (dd, 2H, ^3J = 9.6 Hz, ^5J = 2.4 Hz), 8.62 (d, 2H, ^5J = 2.4 Hz), 10.24 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 34.1, 49.2, 53.3, 54.4, 55.4, 66.4, 98.6, 104.3, 115.5, 116.9, 121.5, 126.7, 133.6, 138.2, 139.2, 161.1, 170.3, 176.3; HRMS calcd for $C_{36}H_{44}N_5O_7$[M + H]$^+$658.3241, found 658.3239.

*2.2.2.7. 2,7-Bis[3-(4-hydroxyl-piperidino)propionamido]-10-(3,5-dimethoxybenzyl)-9, 10-dihydro-acridone (**6g**).* Yield 114 mg, 83%; m.p. 140–142 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.41 (m, 4H), 1.72 (m, 4H), 2.08 (m, 4H), 2.48 (m, 4H), 2.63 (m, 4H), 2.75 (m, 4H), 3.44 (m, 2H), 3.66 (s, 6H), 4.54 (m, 2H), 5.69 (s, 2H), 6.26 (m, 2H), 6.41 (m, 1H), 7.60 (m, 2H), 7.91 (m, 2H), 8.60 (m, 2H), 10.33 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 34.6, 34.7, 49.2, 50.9, 54.0, 55.4, 66.5, 98.6, 104.3, 115.4, 116.9, 121.5, 126.6, 133.6, 138.1, 139.2, 161.1, 170.5, 176.3; HRMS calcd for $C_{38}H_{48}N_5O_7$[M + H]$^+$686.3554, found 686.3553.

*2.2.3. General preparation of compound **8***

To a solution of Boc-amino acid (1.2 mmol) in dry THF (10 mL) was added HOBt (162 mg, 1.2 mmol), DIC (152 mg, 1.2 mmol) and 2,7-diamino-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridone **4** (187 mg, 0.5 mmol). The reaction suspension was stirred under nitrogen overnight at room temperature. The volatile parts were removed under reduced pressure and compound 7 was obtained by column chromatography. Compound **7** was suspended and stirred in hydrochloride 1,4-dioxane solution (15 ml), The suspension was stirred at room temperature until TLC indicated completion of reaction. Yellow solids were obtained after filtration.

*2.2.3.1. 2,7-Bis-(2-ammonium-methyl-carbamoyl)-10-(3,5-dimethoxybenzyl)-9,10-di-hydro-acridoneBis-hydrochlorate (**8a**).* Yield 140 mg, 57%; yellow solids; m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.66 (s, 6H), 3.84 (s, 4H), 5.72 (s, 2H), 6.28 (m, 2H), 6.42 (m, 1H), 7.68 (m, 2H), 7.95 (m, 2H), 8.31 (brs, 6H), 8.68 (m, 2H), 11.02 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 41.2, 49.3, 55.4, 98.6, 104.3, 115.8, 117.3, 121.5, 126.7, 132.7, 138.7, 139.1, 161.2, 165.0, 176.2; HRMS calcd for $C_{26}H_{28}N_5O_5$[M-HCl$_2$]$^+$490.2090, found 490.2087.

*2.2.3.2. 2,7-Bis-(3-ammonium-ethyl-carbamoyl)-10-(3,5-dimethoxybenzyl)-9,10-di-Hydro-acridoneBis-hydrochlorate (**8b**).* Yield 155 mg, 60%; yellow solids; m.p. 262–264 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.78 (t, 4H, ^3J = 6.8 Hz), 3.12 (m, 4H), 3.66 (s, 6H), 5.70 (s, 2H), 6.26 (m, 2H), 6.41 (m, 1H), 7.63 (m, 2H), 7.90–7.94 (m, 8H), 8.70 (m, 2H), 10.49 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 33.4, 35.2, 49.1, 55.4, 98.6, 104.3, 115.8, 117.0, 121.5, 126.8, 133.3, 138.3, 139.1, 161.1, 168.6, 176.3; HRMS calcd for $C_{28}H_{32}N_5O_5$[M-HCl$_2$]$^+$518.2403, found 518.2393.

*2.2.3.3. 2,7-Bis-(4-ammonium-propyl-carbamoyl)-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridoneBis-hydrochlorate (**8c**).* Yield 166 mg, 61%; yellow solids; m.p. 267–269 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.92 (m, 4H), 2.50 (m, 4H), 2.87 (m, 4H), 3.66 (s, 6H), 5.71 (s, 2H), 6.26 (m, 2H), 6.42 (m, 1H), 7.62 (m, 2H), 7.96 (m, 2H), 8.05 (brs, 6H), 8.67 (m, 2H), 10.43 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.8, 32.7, 38.8, 48.7, 54.9, 98.1, 103.9, 115.2, 116.4, 121.0, 126.3, 133.1, 137.8, 138.7, 160.7, 170.1, 175.9; HRMS calcd for$C_{30}H_{36}N_5O_5$[M-HCl$_2$]$^+$546.2716, found 546.2725.

*2.2.3.4. 2,7-Bis-(5-ammonium-butyl-carbamoyl)-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridoneBis-hydrochlorate (**8d**).* Yield 172 mg, 60%; yellow solids; m.p. 199–201 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.62–1.68 (m, 8H), 2.40 (m, 4H, ^3J = 6.8 Hz), 2.81 (m, 4H), 3.66 (s, 6H), 5.70 (s, 2H), 6.25 (m, 2H), 6.41 (m, 1H), 7.61 (m, 2H), 7.93–7.95 (m, 8H), 8.67 (m, 2H), 10.32 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 22.6, 27.0, 36.1, 39.0, 49.3, 55.6, 98.8, 104.6, 115.8, 117.1, 121.7, 127.0, 133.9, 138.4, 139.5, 161.4, 171.4, 176.6; HRMS calcd for $C_{32}H_{40}N_5O_5$[M-HCl$_2$]$^+$574.3029, found 574.3028.

*2.2.3.5. 2,7-Bis-(2-ammonium-ethyl-carbamoyl)-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridoneBis-hydrochlorate (**8e**).* Yield 137 mg, 53%; yellow solids; m.p. 252–253 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52 (d, 6H, ^3J = 6.8 Hz), 3.66 (s, 6H), 4.14 (m, 2H), 5.73 (s, 2H), 6.27 (m, 2H), 6.42 (m, 1H), 7.68 (m, 2H), 7.99 (m, 2H), 8.43 (brs, 6H), 8.71 (m, 2H), 11.11 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 17.7, 49.4, 49.5, 55.6, 98.9, 104.6, 116.4, 117.5, 121.7, 127.1, 133.0, 138.9, 139.3, 161.4, 168.7, 176.4; HRMS calcd for $C_{28}H_{32}N_5O_5$[M-HCl$_2$]$^+$518.2403, found 518.2402.

*2.2.3.6. 2,7-Bis-(2-ammonium-3-methyl-propyl-carbamoyl)-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridoneBis-hydrochlorate (**8f**).* Yield 172 mg, 60%; yellow solids; m.p. 242–244 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.02–1.04 (m, 12H), 3.66 (s, 6H), 3.90 (m, 1H), 5.73 (s, 2H), 6.27 (m, 2H), 6.43 (m, 1H), 7.69 (m, 2H), 7.99 (m, 2H), 8.41 (brs, 6H), 8.71 (m, 2H), 11.20 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 18.2, 18.7, 30.2, 49.2, 55.4, 59.2, 98.5, 104.3, 116.2, 117.3, 121.5, 126.9, 132.5, 138.8, 139.0, 161.2, 167.0. 176.2; HRMS calcd for $C_{32}H_{40}N_5O_5$[M-HCl$_2$]$^+$574.3029, found 574.3026.

2.3. Measurement of G-quardruplex binding ability

2.3.1. Circular dichroism spectra

The CD spectra of DNA oligonucleotides 10 μM $(TTAGGG)_4$ with 0, 20 and 40 μM **6a** in NH_4OAc buffer were carried out at room temperature by using a J-815 spectropolarimeter (JASCO) with a 0.1 cm path-length quartz cell. The CD spectrum was scanned three times and obtained by taking the average of them. The scan for buffer was subtracted from the average scan each time.

2.3.2. Absorption spectra

20 μM compound **6a** were incubated in NH_4OAc buffer (pH 7.4) in the presence or absence of 7 or 14 μM of the G-quadruplex DNA. UV–Vis absorption spectra were all recorded using DU 800 spectrophotometer (BeckmanCoulter, Atlanta, GA, USA) with a quartz cell having 1.0 cm pathway.

2.3.3. Mass spectra

The mass spectra were acquired using a Waters Q-Tof Premier mass spectrometer equipped with an electrospray ionization (ESI) source. The instrument was operated in the negative-ion mode. Compound **6a** and DNA solution was diluted with 20:80 (v/v) methanol/100 mm ammonium acetate. The binding assays were performed at 25 μM DNA and compound **6a** (25 μM). Methanol was added to obtain a good spray [29]. The direct infusion flow rate was 10 μl/min. The electrospray source conditions were spray voltage of 2.4 kV and capillary temperature of 120 °C [30].

2.3.4. FRET assay

The labeled oligonucleotides F21T [5′-FAM-d(GGG[TTAGGG]3)-TAMRA-3′] were purchased from invitrogen (Guangdong, China) and the FRET assay was carried out on a real-time PCR apparatus (Roche LightCycler 2). The FRET probes were diluted to the correct concentration (200 nM) in Tris–HCl buffer (10 mM, pH 7.4) containing 60 mM KCl. Compound **6a** was diluted to concentrations of 1.0, 1.4, and 2.0 μM, respectively. Samples were incubated for 1 h. and then put into LightCycler capillaries. Measurements were made in triplicate on a RT-PCR with excitation at 470 nm and detection at 530 nm. Fluorescence readings were taken at intervals of 1 °C over the range 37–99 °C, with a constant temperature being maintained for 30 sprior to each reading to ensure a stable value.

2.4. In-vitro cytotoxic activity evaluation by MTT assay

MTT assays were performed according to the methods reported by our previous papers [27,28,31–33].

3. Results and discussion

3.1. Chemistry

The synthetic route of desired compound is similar to our previous papers [27]. As shown in Scheme 1, starting from acridone, 2,7-diamino-l0-(3,5-dimethoxy)benzyl-9(10H)-acridone **4** was obtained by three-stage reaction. Acylation reaction of compound **4** with 3-chloropropionyl chloride yielded the 2,7-bis(3-Chloropropionamido)-10-(3,5-dimethoxybenzyl) 9,10-dihydro-acridone

5 in high yields. The followed nucleophilic displacement of the chloride by the appropriate amine in the presence of KI gave the desired compounds **6(a–g)** in good yields. Compounds **8(a–f)** were synthesized according to the reference [28], as seen in Scheme 2 and the above Experimental section. All synthesized new compounds were characterized pure by ^{1}H NMR, ^{13}C NMR and high resolution mass spectral data.

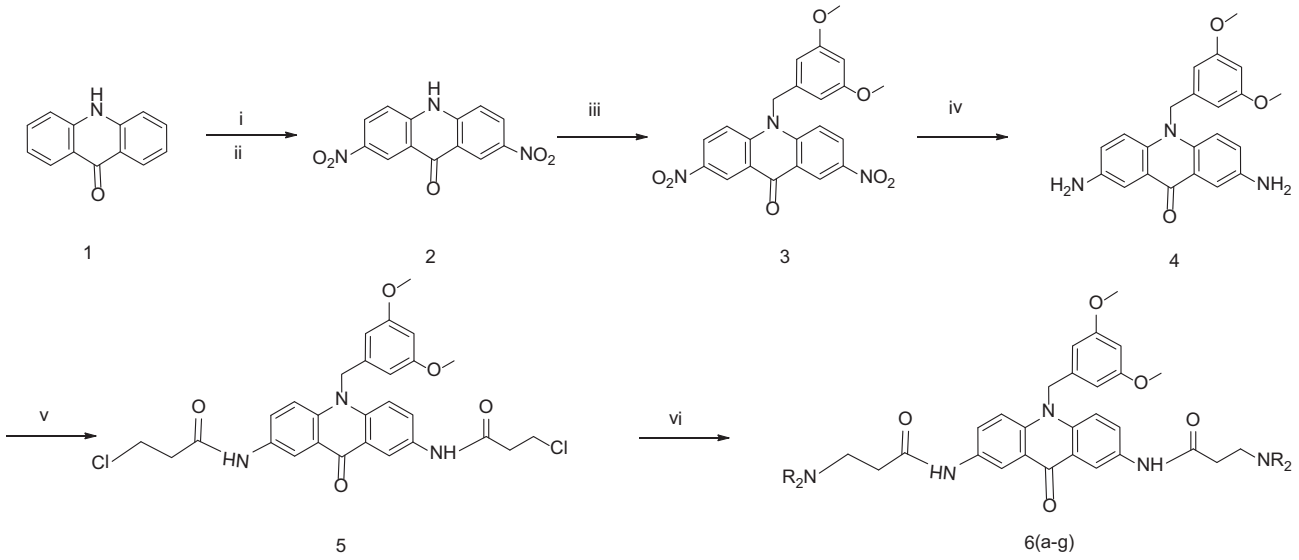
3.2. Cytotoxic activity

Two series of synthesized new l0-(3,5-dimethoxy)benzyl-9(10H)-acridone derivatives with a range of side chain substituents at C2 and C7 position on the acridone ring were tested for their antiproliferative activity against leukemia CCRF-CEM using 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT) assay after 48 h of treatment with increasing concentrations of the tested compounds. Etoposide was used as the positive control. The results were shown in Table 1.

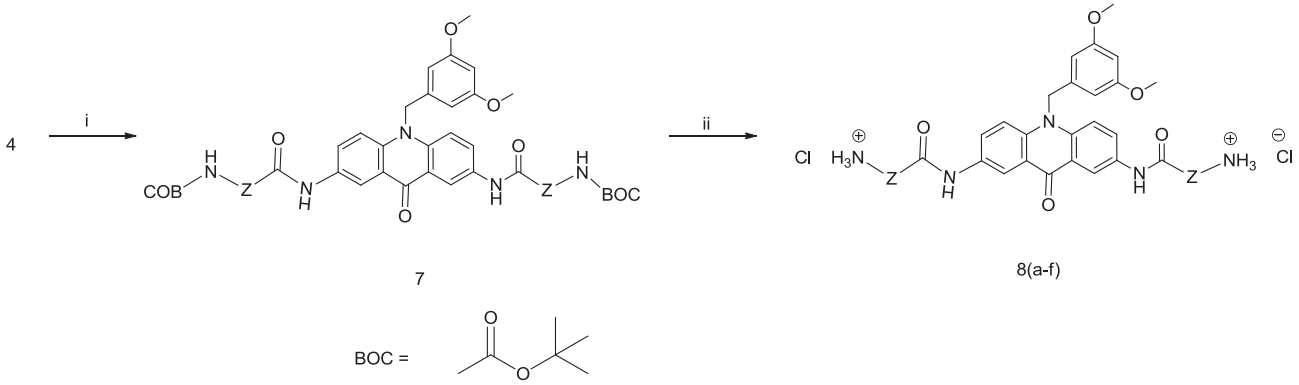
Compound **6(a–g)** with tertiary amines at the termini of the chains displayed moderate to good antiproliferation activity, some of which had IC_{50} values at a low micromolar range. Compound **6a** containing dimethylamine substituents was considered as the most potent antiproliferation agent with an IC_{50} at 0.3 μM. By changing dimethylamine group to diethylamine and diethanol amine substituents, compounds **6b** and **6c** showed decreasing inhibitory effect. Compound **6b** was determined to be 15-fold less potent than **6a**. **6c** was determined to be 8-fold less potent than **6b**. In addition, compounds bearing terminal cyclic amino moiety (**6d–6**) were also evaluated, among which compound **6e** with piperidine groups displayed the highest antiproliferative activity with an IC_{50} at 2.3 μM. The replacement of piperidine to 4-hydroxyl piperidine reduced about 10-fold cytotoxicity. Derivatives with pyrrolidine (**6d**) and morpholine (**6f**) groups were essentially inactive. The data indicated that the pattern of tertiary amines played important roles on the cytotoxicity.

Compared with the antiproliferative activity of **6a–6g**, analogues **8** displayed lower activity. Compounds **8a–8d** with different length of the alkyl chain on the amino acid moiety were inactive. By changing the methylene group (**8a**) to methyl methylene (**8e**) or isopropyl methylene (**8f**), compounds showed moderate inhibition effect against CCRF-CEM cells.

As compound **6a** displayed the highest activity against CCRF-CEM cells, to further investigate its antiproliferative potential and toxicity against different cancer cells and normal cells, the cytotoxic activity against A549, MCF7, 293T, K562, and HCT-116 cells was evaluated. As shown in Table 2, compound **6a** possessed little activities against the growth of the three solid tumor cell lines. The results indicated that **6a** had high selectivity to leukemia cells with

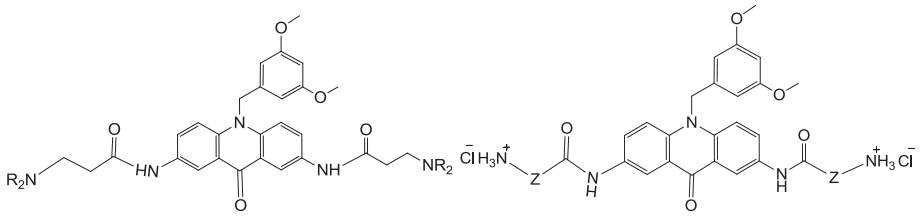


Scheme 1. Reagents and conditions (i) HNO_3, HOAC (ii) HNO_3, H_2SO_4 (iii) 3,5-dimethoxybenzyl chloride, NaH (iv) $Na_2S{\cdot}9H_2O$, C_2H_5OH (v) chloropropionyl chloride, THF (vi) R_2NH, EtOH, KI, reflux.

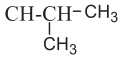
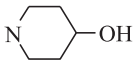


Scheme 2. Reagents and conditions (i) HOBt, DIC (ii) HCl in 1,4-dioxane.

Table 1
Chemical data and antiproliferative activity against CCRF-CEM cells of compound **6a–6g** and **8a–f**.



6a-6g **8a-8h**

Compound	*R*	IC_{50} (μM)	Compound	*Z*	IC_{50} (μM)
6a		0.3 ± 0.1	8a	CH_2	>50
6b		4.4 ± 0.5	8b	$(CH_2)_2$	>50
6c		35.5 ± 3.2	8c	$(CH_2)_3$	>50
6d		>50	8d	$(CH_2)_4$	>50
6e		2.3 ± 0.2	8e		30.9 ± 0.8
6f		>50	8f		9.7 ± 0.8
6g		26.1 ± 2.2	Etoposide		0.14 ± 0.03

no toxic effects on normal 293T cells, which made it a good candidate for further development.

Table 2
IC_{50} values (μM) ofcompound **6a** against A549, MCF-7, 293T, HCT-116 and K562 cell lines.

	A549	MCF-7	293T	HCT-116	K562
6a	>50	>50	>100	21.3 ± 2.6	4.7 ± 0.2

3.3. G-quadruplex DNA binding ability

3.3.1. UV–Vis absorption spectra

Generally, when acridine or acridone derivatives intercalated into DNA, the stacking interaction between aromatic chromophore and the base pairs of DNA will lead to bathochromic shifts and hypochromicity on the UV–Vis curve of compound-DNA complex [34–36]. To evaluate the G-quadruplex DNA binding capacity of our compounds, the representative spectra of **6a** in the absence and presence of G-quadruplex DNA were investigated. As shown

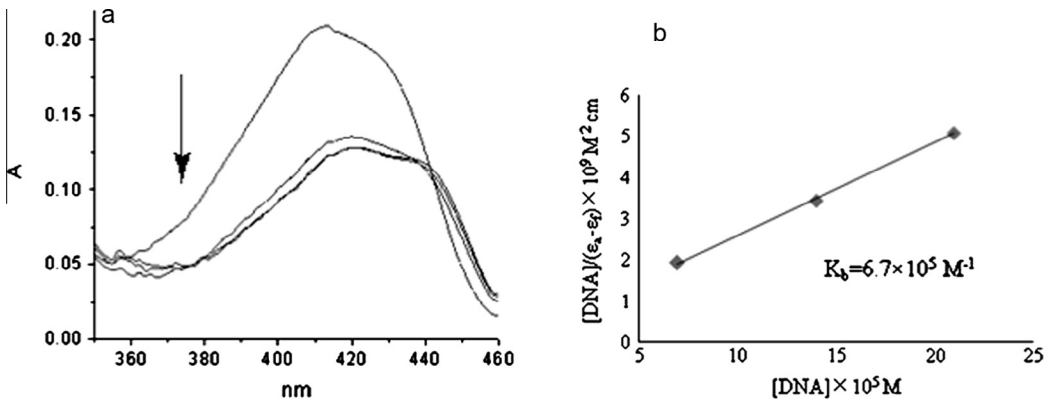


Fig. 1. (a) UV–Vis absorption spectra of 20 μM **6a** with 0, 7, 14, 21 μM G-quadruplex DNA in NH_4OAc buffer. DNA Vertical arrows indicate the increase of the absorbance change upon increasing DNA concentration. (b) The plot of [DNA]/($\varepsilon_a-\varepsilon_f$) as a function of DNA concentration determined from the absorption spectral data.

in Fig. 1a, compound **6a** showed the maximal absorption around 414 nm with an absorption bands in the region of 360–460 nm, while the maximal absorption is about 420 nm in the presence of G-quadruplex DNA. The absorption spectra also demonstrated significant hypochromicities. Both the bathochromic shifts and hypochromicity indicated that compound **6a** had the ability to bind G-quadruplex DNA.

In order to further elucidate the G-quadruplex DNA binding ability of **6a**, the binding constant K_b was determined by monitoring the changes in absorbance with increasing concentrations of G-quadruplex DNA. The binding constant K_b was calculated by the ratio of slope to the intercept [37] (Fig. 1b), which was derived to be $6.7 \times 10^5\ M^{-1}$. In addition, the binding ability of compound **6a** to duplex ctDNA was also investigated ($K_b = 1.3 \times 10^5\ M^{-1}$), which was shown in Fig. 1s (Supplementary material). The results indicated that **6a** displayed moderate binding selectivity toward G-quadruplex DNA.

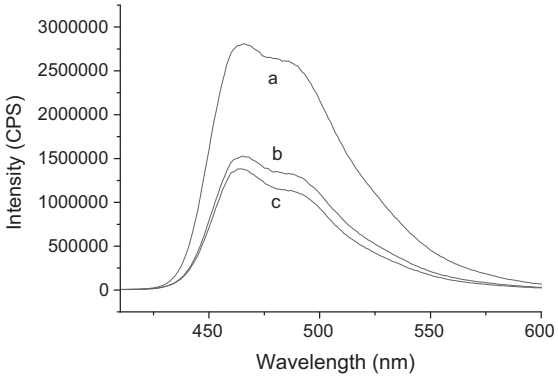


Fig. 2. Fluorescence emission spectra of 20 μM acridone derivatives **6a** with various concentrations of G-quadruplex DNA in NH_4OAc buffer after excitation at λex = 380 nm. (a) 0 μM G-quadruplex DNA; (b) 10 μM G-quadruplex DNA; (c) 20 μM G-quadruplex DNA.

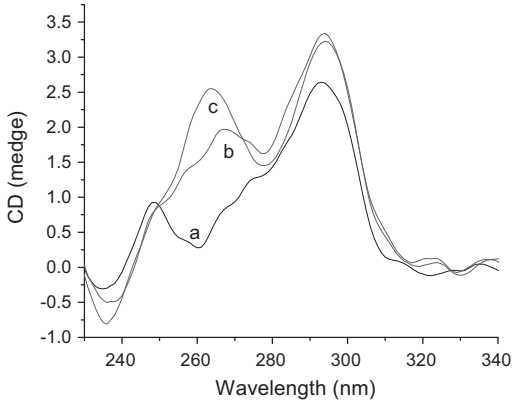


Fig. 3. CD spectra of 10 μM $(TTAGGG)_4$ with various concentrations of **6a** in NH_4OAc buffer: (a) 0 μM **6a**; (b) 20 μM **6a**; (c) 40 μM **6a**.

3.3.2. Fluorescence emission spectra

The fluorescence of acridine or acridone derivatives always quenched severely after forming compound-DNA complex. In order to gain further insight into the binding properties of the human telomeric G-quadruplex DNA with **6a**, the fluorescence spectra in NH_4OAc aqueous solution had been recorded in the absence and presence of G-quadruplex DNA. About 50% decrease of the peak intensity in the presence of DNA was observed (Fig. 2). This result can be served as another proof of the interaction between compound **6a** and G-quadruplex DNA.

3.3.3. Circular dichroism spectroscopy

Circular dichroism (CD) has been used as a powerful tool to monitor the change of biomacromolecules for years due to its outstanding sensitivity. To further confirm the interaction mode between **6a** and G-quadruplex DNA, CD spectra of free G-quadruplex in the absence and presence of **6a** were studied (Fig. 3). The CD spectrum of free G-quadruplex DNA had a negative peak near 235 nm, a small positive peak at about 250 nm and a positive peak near 295 nm associated with a 274 nm positive shoulder in NH_4OAc buffer, which indicated that the coexistence of antiparallel, parallel G-quadruplex structure and other hybrid forms. After the addition of **6a**, a strong enhancement in the CD intensity at both 265 and 295 nm, which suggested that **6a** could increase both the parallel and antiparallel structures.

3.3.4. Mass spectrometry

Electrospray ionization mass spectrometry (ESI–MS) is a highly sensitive method to investigate the binding ability of compounds and DNA [38,39]. Therefore, the binding of compounds to human G-quadruplex DNA was analyzed by ESI–MS. In the NH_4OAc buffer, the ESI–MS spectrum of the DNA revealed two main ions at m/z 1262 and 1514 confirming the formation of the quadruplex (Fig. 4). When compound **6a** was added to the G-quadruplex DNA solution, a mixture of 1:1 and 2:1 drug-DNA complexes were identified. These indicated that the compound **6a** was a suitable binder for human telomeric quadruplex DNA.

3.3.5. FRET assays of quadruplexbinding

The ability of compound **6a** to stabilize human telomeric quadruplex DNA was also evaluated by fluorescence resonance

Table 3
Thermal stabilization of G-quadruplex DNA for compound **6a** at different concentrations.

6a (μM)	1.0	1.4	1.6	2
ΔT_m (°C)	10	14	23	28

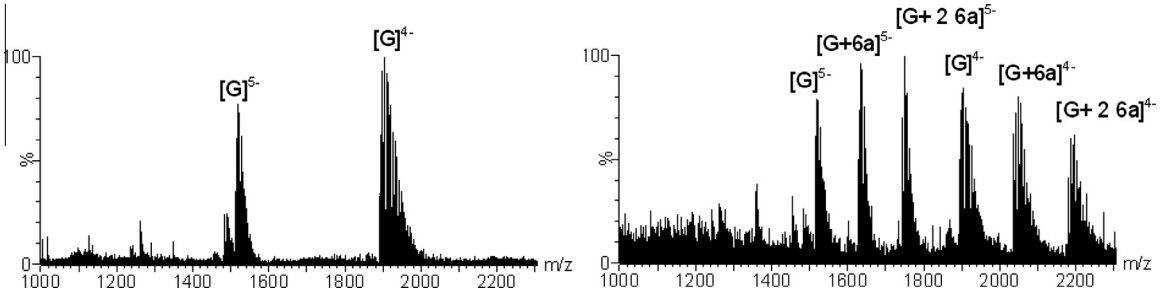


Fig. 4. (a) ESI MS spectra of 25 μM G-quadruplex DNA and (b) compounds **6a** with G-quadruplex DNA in a 1:1 M ratio in NH_4OAc buffer.

energy transfer (FRET) methods. The data in Table 3 showed that compound **6a** had great ability to stabilize G-quadruplex DNA. As the concentration of **6a** increased from 1.0 μM to 2.0 μM, the ΔT_m value increased from 10 to 28 °C. This confirmed again that **6a** had the ability to be developed as potent G-quadruplex DNA stabilizers.

4. Conclusions

We have prepared a series of l0-(3,5-dimethoxy)benzyl-9(10H)-acridone derivatives with terminal ammonium substituents at C2 and C7 positions on the acridone ring and evaluated their antiproliferative activity. The representative compound **6a** displayed high antiproliferation activity and little toxic activity against normal cells, which might be associated with its stabilization of G-quadruplex DNA. The further structure optimization is in progress.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2015.04.002.

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