Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 1356–1362
Bioorganic & Medicinal Chemistry

Novel antitumor agent family of 1*H*-benzo[*c*,*d*]indol-2-one with flexible basic side chains: Synthesis and biological evaluation

Hong Yin, Yufang Xu and Xuhong Qian*

Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China, University of Science and Technology, Shanghai 200237, China

Received 8 October 2006; revised 4 November 2006; accepted 6 November 2006
Available online 11 November 2006

Abstract—A series of mono-1*H*-benzo[*c*,*d*]indol-2-one with different amine side chains and bis-1*H*-benzo[*c*,*d*]indol-2-one as novel family of DNA intercalators were designed and synthesized, the contributions of aromatic chromophores and amine side chains for DNA binding properties, for example, intercalation and electrostatic binding, respectively, were evaluated. Among them, **A3** tailed with *N*,*N*-dimethylamino-ethyl-ethane-1,2-diamine showed selective anti-tumor activities against cell lines A549 and P388 with IC_{50} 0.428 μm and 1.69 μm.

1. Introduction

The discovery of new compounds with antitumor activity became one of the most important goals in medicinal chemistry. DNA-targeted chemotherapeutic agents were the mainstay for cancer therapy.[1,2] Most of DNA's interacting binders were characterized by the presence of a planar chromophore, generally a tri- or tetracyclic ring system with flexible basic side chains. The rationale was mostly based on that the planar aromatic rings such as naphthalimides, pyridocarbazole, acridine, anthracyclines, benzimidazo[1,2-*c*]quinazoline, and so on provided DNA binding affinity,[3,4] the basic side chains with multitude of cationic site charges supported the DNA sequence selectivity and allowed the molecule to adjust its structure to follow the groove, additionally contributing to interference with topoisomerases and perhaps facilitating the cellular penetration of the molecule.[5] Some meaningful examples were amonafide (**a**), elinafide (**b**),[6] SN 16713 (**c**), and DACA (**d**) (Fig. 1).[7]

Naphthostyril (**e**) derivatives played an important role not only in good binding affinity for Thymidylate Synthase (TS) but also favorable inhibitor against them especially human TS.[8,9] The region of the lactam group on the tricyclic rings could form hydrogen bond[9,10] with the active site of TS, and their interaction was thought to contribute to the binding. On the basis of the similarity between naphthostyril or benzo[*c*,*d*]indol-2-one and naphthalimide as famous DNA intercalative antitumor, we wondered if the lactam group could interact with DNA as well through the hydrogen bond between the lactam oxygen and the backbone of nucleic acids and also acted as intercalating chromophore moiety, which was required for useful antitumor activity (Fig. 1).

Meanwhile, it is well known, *N*,*N*-dimethyl ethyldiamino group and its analogues commonly applied in anticancer agents[11] conjugated into the intercalating

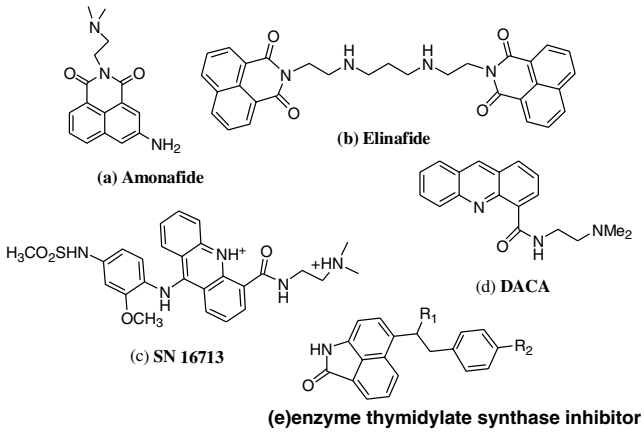

Figure 1. Structures of some reported compounds.

* Corresponding author. Tel.: +86 021 64253589; fax: +86 021 64252603; e-mail: xhqian@ecust.edu.cn

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.11.016

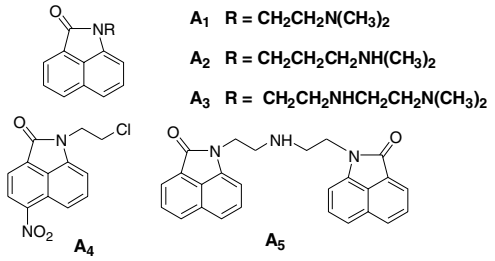

Figure 2. Structures of the novel potential antitumor compounds.

moiety as the basic side chain. Therefore, we designed two novel compounds **A1** and **A2** as potential anticancer agents. Moreover, in recent years, much attention had been paid to polyamines, which were positively charged and present in prokaryotic and eukaryotic cells.[12] The polyamine cations had 'high' DNA affinity but were 'loosely' bound and could 'read' DNA.[13] At molecular level, it could modulate DNA conformation.[14,15] Furthermore, the intercalator conjugated with polyamine cations had been reported to have better antitumor activity and selectivity,[16,11d] so compound **A3** was designed as new kind of potential antitumor agents. Additionally, we were interested in bis-intercalators by linking two planar rings with a linkage such as Elinafide, and the compound **A5** was therefore designed. In order to find a novel hybrid compound, we also synthesized 1-(2-chloro-ethyl)-5-nitro-1*H*-benzo[*c*,*d*]indol-2-one (**A4**), which would be monocrosslinked with DNA as alkylating agents and resulted in inhibition of tumor growth.[2] Their spectra, anti-tumor properties, and DNA-binding affinities are also studied herein (Fig. 2).

2. Results and discussion

2.1. Synthesis and spectral data

The synthetic route is shown in Figure 3. 1*H*-Benzo[*c*,*d*]indol-2-one was reacted at refluxing for 5 h with 1,2-dibromoethane and 1,3-dichloropropane, respectively, in acetonitrile in presence of K_2CO_3 as catalyst to give intermediates (**1**) and (**2**), then (**1**) was reacted with dimethylamine solution and *N*,*N*-dimethylethylenediamine to give the target compounds **A1** and **A3**. (**2**) was reacted with dimethylamine solution to afford compound **A2**. 1*H*-Benzo[*c*,*d*]indol-2-one was stirred in concentrated sulfuric acid at 0–5 °C with HNO_3 in concentrated sulfuric acid added drop-wise and then stirred at room temperature to give **3**, then the solid was refluxed in 1,2-dichloroethane to produce **A4**. Diethanolamine in chloroform was added to $SOCl_2$ in chloroform drop-wise at 0–5 °C, then stirred at room temperature to afford dichloroethylamine. The amine reacted with 1*H*-benzo[*c*,*d*]indol-2-one to give **A5**. Structures of all these final products were identified by ^{1}H NMR, HRMS, and IR.

The aminoalkyl side chains were found to have slight effect on the UV–vis and fluorescent spectra of **A1**–**A3** (Table 1). Their maximal absorptions were around 336 nm with different intensities. **A5** showed the strongest intensity with 4.08 (log ε), and the maximal emissions were at 496 nm because of the two chromophores in the molecule. All of the compounds had low quantum efficiency.

2.2. DNA interaction property

The binding properties between the compounds and ctDNA were evaluated. With titration of ctDNA, the mixed solutions were incubated at 25 °C for 6 h, then the UV–vis absorption and the fluorescent spectra were measured, and the representative curves for compounds **A1** and **A3** are illustrated in Figures 4 and 5, respectively.

In Figure 4, representative UV–vis spectra for compounds **A1** and **A3** are shown in (a) and (b), in which spectra 1 and 2 corresponded to their UV–vis spectra before and after the addition of ctDNA at the DNA/**A3** molar ratio of 2.0, respectively. It was observed that the addition of ctDNA to the solution of **A1** induced bathochromic shifts (1–7 nm) and hypochromicities (4.8–10%), the bathochromic shifts (1–9 nm) and strong hypochromicities (7.1–19.4%) were observed in the spectra (b) of Figure 4, which showed **A3** binding ctDNA with higher affinity in the UV–vis absorption spectra.[17] On the other hand, for **A1** there was only one isosbes (308 nm) in the UV–vis absorption, however for **A3** three obvious isosbestic points at around 318, 409, and 545 nm were observed, which indicated that there were different equilibriums for **A1** and **A3** between binding and free drug. The reasons were probably due to the fact that the different cationic side chains of **A1** and **A3** resulted in different charge-charge attraction with the phosphate backbone of the DNA.

From the analyses of the relationship between the fluorescence intensities and the DNA concentrations by nonlinear curve fitting methods, these significant changes make the association steady constants of **A1** and **A3** with DNA available with $3.0 \times 10^4\ M^{-1}$ and $5.7 \times 10^4\ M^{-1}$, respectively,[18] which indicated that **A3** was more stable than **A1** in binding to ctDNA.

From (a) in Figure 5, it is found that the fluorescence of **A1** was quenched as most intercalators did with the addition of increasing ctDNA concentration, and **A3** exhibited enhanced intensities as (b) in Figure 5. It was also very interesting that, for **A3** when it was incubated with ctDNA from 2 h to 6 h as shown in Figure 5(c), the fluorescence was quenched first, then began to enhance. In order to provide further insight into the special interaction, the fluorescence spectra of the mixture were evaluated along with the increasing time as shown in Figure 6. From (b) in Figure 6, it was found that the fluorescence of **A3** + DNA1 (10:1) was quenched after incubated for 1 h, 2 h, then kept almost constant, and the fluorescence began to enhance 4 h later. The sample **A3** + DNA2 (1:10) had almost the same trend as shown in Figure 6(c), which indicated that the interaction was possibly associated with thermodynamic equilibrium process. Taken the structure into account, the reasons were probably due to the fact that the tri-cyclic ring of

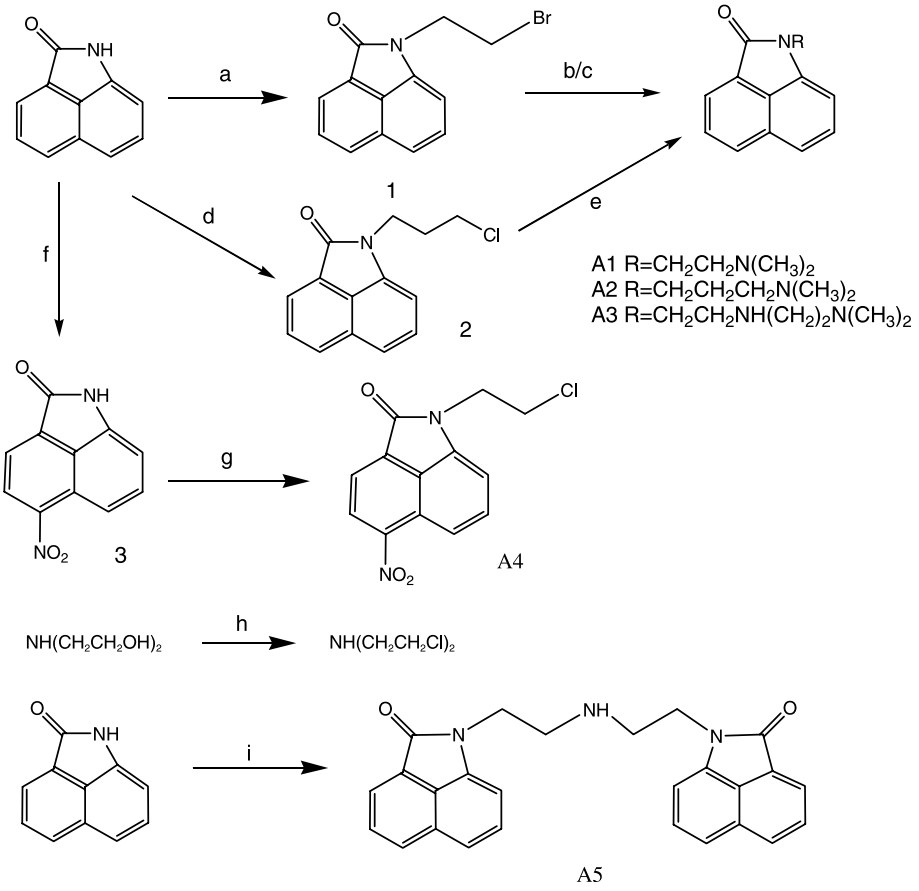

Figure 3. Synthesis of target compounds. Reagents and conditions: (a) $BrCH_2CH_2Br$, acetonitrile, K_2CO_3, 81% yield; (b) dimethylamine solution, reflux, 54% yield; (c) *N*,*N*-dimethylethylenediamine, acetonitrile, 68% yield; (d) $ClCH_2CH_2CH_2Cl$, acetonitrile, 53% yield; (e) dimethylamine solution, reflux, 44% yield; (f) HNO_3, H_2SO_4, ice bath, 85% yield; (g) $ClCH_2CH_2Cl$, acetonitrile, K_2CO_3, 65%; (h) $SOCl_2$, $CHCl_3$, 82% yield; (i) K_2CO_3, DMF, reflux, 10 h, 49% yield.

Table 1. Spectra data[a,b] of **A1**–**A5**

Compound	UV λ_{max}/nm (lg ε)	FL λ_{max}/nm (Φ)	Excitation wavelength λ/nm
A1	336.3 (3.60)	490.7 (0.0016)	336.3
A2	336.3 (3.94)	495.6 (0.0015)	336.3
A3	336.3 (3.56)	492.5 (0.0011)	336.3
A4	336.5 (2.57)	493.7 (0.0003)	336.5
A5	337.2 (4.08)	496.1 (0.0014)	337.2

[a] In absolute ethanol.
[b] With quinine sulfate in sulfuric acid as quantum yield standard (Φ = 0.55).

A3 was intercalated into base pair at first, then the long and flexible polyamine side chain continued to modulate itself to interact with ctDNA in an apropos conformation. Because the polyamine interacted readily with the DNA phosphate anionic backbone, causing condensation by charge neutralization, this effect was also a key first step in minimizing the size of foreign DNA for gene therapy.[19] On the other hand, Gershon et al.[20] demonstrated that at high and low charge ratios, the fluorescence intensity was at its extreme values. However, at the key intermediate charge ratios during the DNA condensation process, fluorescence was time dependent. Our results also proved that with moderate polyamine length, the fluorescence of the compounds with ctDNA was time dependent.

2.3. Damage of plasmid DNA property

In an effort to find further biological properties, we explored the damage of plasmid DNA property for **A1**–**A5**, on the basis that small molecules characterized with a DNA intercalator tailed with dimethylamino alkyldiimide could cleave supercoiled plasmid DNA.[21] Herein, Agarose gel electrophoresis was carried out at 70 °C for 4 h as shown in Figure 7.

Figure 7 shows that all of the compounds could cleave the closed supercoiled DNA but with low efficiency. **A3** was found to have better ability than others, which indicated that the polyamine side chain of **A3** probably contributed favorable biological activities.

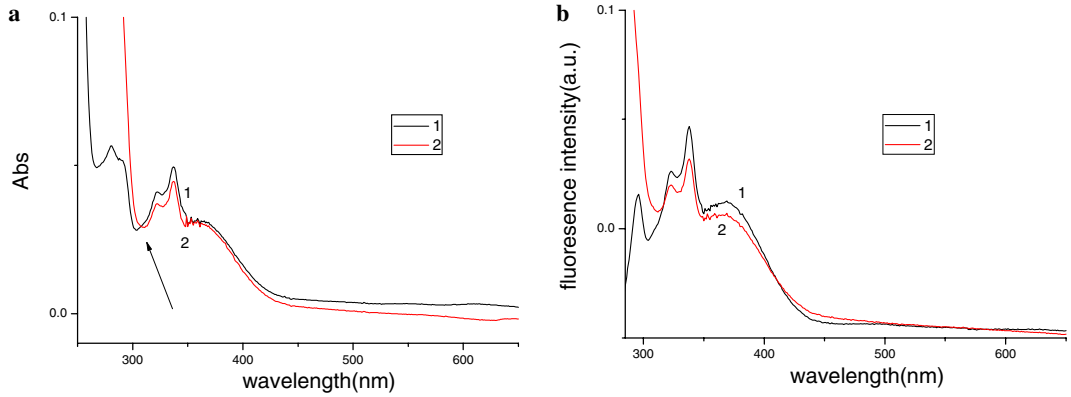

Figure 4. UV–vis absorption spectra of **A1** (a), **A3** (b) (10 μM, 25 °C, 6 h), in 20 mM Tris–HCl buffer (pH 7.5) before (1) and after (2) the addition of ctDNA. The concentration ratio of DNA/drug was 2:1. The arrows indicate the isosbes.

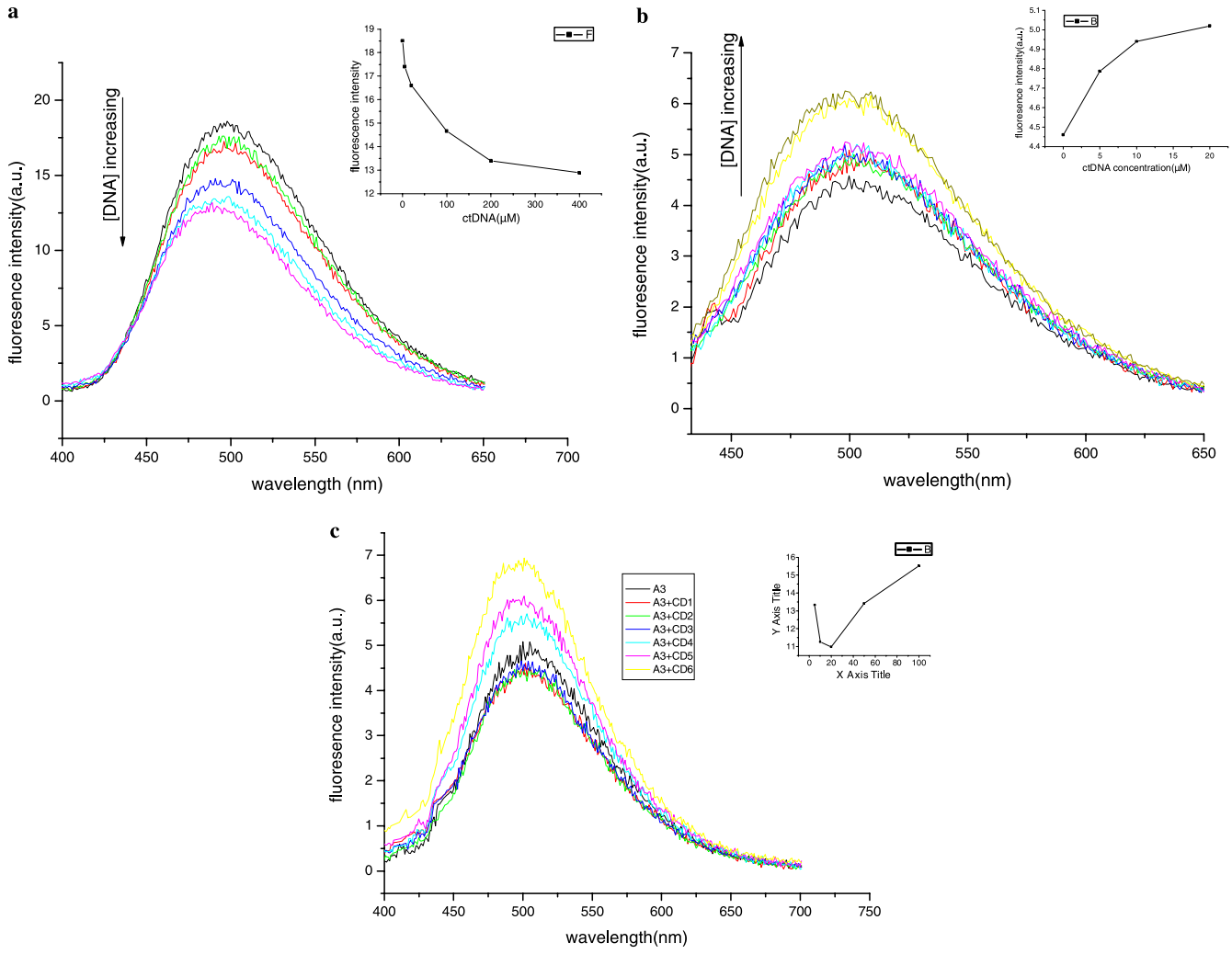

Figure 5. Fluorescence spectra of (a) **A1** (6 h) and (b) **A3** (6 h); (c) **A3** (2 h) (10 μM) with ctDNA. The concentrations of ctDNA were increased from 0 to 400 μM in 20 mM Tris–HCl (pH 7.5) at 25 °C. The insets indicated the relationship between the fluorescent intensity and the concentration of ctDNA (μM).

2.4. Antitumor evaluation

The antitumor activities of compounds **A1**–**A5** were evaluated in vitro against cell lines A549 (human lung cancer cell) and P388 (murine leukemia cell), respectively, listed in Table 2. The IC_{50} represents the drug concentration (μM) required to inhibit the cell growth by 50%.

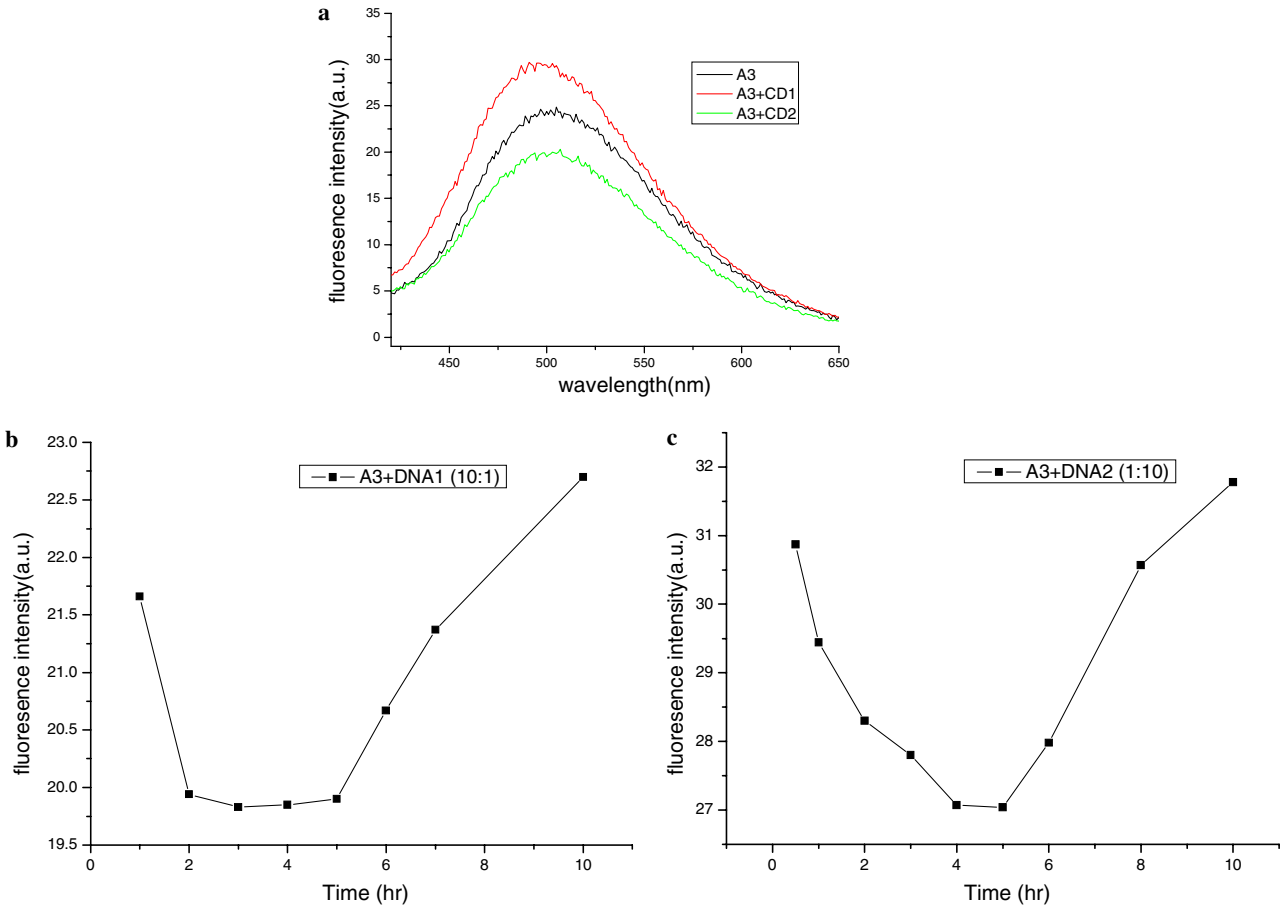

Figure 6. Fluorescence spectra of (a) **A3** before and after the addition of ctDNA: **A3** + DNA1 (1:10), **A3**, **A3** + DNA2 (10:1) incubated at 25 °C for 2 h. (b) **A3** + DNA1 incubated for 1–10 h in 20 mM Tris–HCl (pH 7.5) at 25 °C. (c) **A3** + DNA2 incubated for 1–10 h in 20 mM Tris–HCl (pH 7.5) at 25 °C.

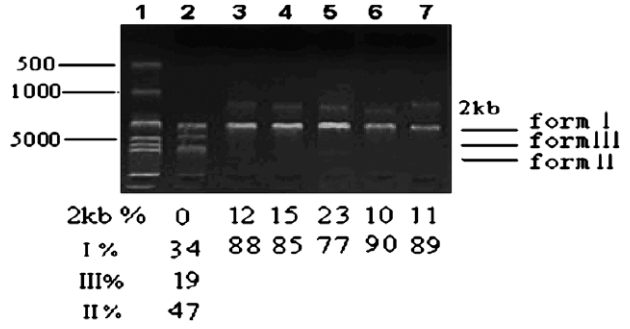

Figure 7. 1% Agarose gel electrophoresis assay of plasmid PBR 322DNA cleaved by compounds **A1**–**A5**. Lane 1, DNA marker; lane 2, 200 ng pBR322 DNA (incubated at 70 °C for 4 h); lanes 3–7, 200 ng pBR322 DNA and **A1**, **A2**, **A3**, **A4**, **A5** (100 μM), respectively, incubated at 70 °C for 4 h in 20 mM Tris–HCl (pH 7.5).

Table 2. Cytotoxicity of compounds **A1**–**A5** against cell lines A549[a] and P388[b]

Compound	Cytotoxicity (IC_{50}, μm)	
	A549	P388
A1	300	79.4
A2	22.9	7.76
A3	0.489	1.69
A4	52.5	17.8
A5	29.5	13.8

[a] Cytotoxicity (CTX) against human lung cancer cell (A549) was measured by sulforhodamine B dye-staining method.[16b]

[b] CTX against murine leukemia cells (P388) was measured by micro-culture tetrazolium–formazan method.[11d]

It is shown in Table 2 that **A1** had low activity, **A2**, **A4**, and **A5** (bis-1*H*-benzo[*c*,*d*]indol-2-one) displayed increased potency. They were found to be more cytotoxic against P388 than A549. On the other hand, **A3** was the strongest growth inhibitor against A549 and P388 with IC_{50} of 0.489 and 1.69 μM, respectively. Interestingly, it was also found that the cytotoxicity of this compound against A549 was higher than P388, reflecting the excellent selectivity for a special human lung cell type. The different antitumor activities showed that the modification of such agents for potent antitumor drugs seemed to be dependent on structures of the amine side chains. Specially, the high antitumor activity of compound **A3**, which was incorporated in additional ethyleneamine groups in the side chain, was probably associated with the polyamine-induced DNA condensation in a suitable conformation, so the selectivity and biological activity of the compounds were improved.[20]

3. Conclusion

In summary, we synthesized a novel class of compounds (**A1**–**A5**) featuring tricyclic ring with different side chains. Their antitumor activities together with DNA binding affinities were evaluated, and interaction property with ctDNA (represented as **A1** and **A3**) had been further studied. Also, their damage of supercoiled DNA property was explored. Among the compounds, **A3** exhibited not only the high antitumor activity and the good selectivity, but also distinctive interaction with ctDNA. This work also provided the influence of polycation side chains on their biological activities, which revealed that could be advantageous modulation for antitumor agents.

4. Experimental

4.1. Materials and methods

All the solvents were of analytical grade. The closed supercoiled pBR322 DNA was purchased from Takara Biotech Co. Ltd (Shanghai). ^{1}H NMR was measured on a Bruker AV-500 spectrometer with chemical shifts reported as parts per million (in acetone-d_6/DMSO-d_6/$CDCl_3$, TMS as an internal standard). Mass spectra were measured on a HP 1100 LC–MS spectrometer. Melting points were determined by an X-6 micro-melting point apparatus and are uncorrected. Absorption spectra were determined on PGENERAL TU-1901 UV–VIS Spectrophotometer.

4.2. Spectrophotometric titration experiments

The concentrations of compounds and calf thymus DNA were 10 μM and 0–400 μM, respectively, in 20 mM Tris–HCl buffer, pH 7.5, and DMSO (1% by volume), the solution in a final volume of 10 ml, which was used for fluorescence-quenching experiments. An equilibrium period of 6h for constant stirring at 25 °C in the dark of the mixed solution was allowed before recording each spectrum. The association constants (K_a's) were derived according to the equation $I = I_0 + \{(I_\infty - I_0/2[Q]_0)\} \times \{([DNA]_0 + [Q]_0 + 1/K_a)^2 - 4[DNA]_0[Q]_0\}^{1/2}\}$, wherein I_0, I, and I_∞ represent the fluorescence intensities of compounds alone, the sample, and DNA totally bound, respectively. $[DNA]_0$ and $[Q]_0$ were the initial analytical concentrations of DNA and the agents, respectively.

4.3. Synthesis

4.3.1. 1-(2-Dimethylamino-ethyl)-1*H*-benzo[*c*,*d*]indol-2-one (A1). 1*H*-Benzo[*c*,*d*]indol-2-one 0.35 g (2 mmol), 1,2-dibromo ethane 2.5 ml (0.03 mol), and potassium carbonate anhydrous 0.05 g were added to acetonitrile 20 ml, the mixture was stirred and refluxed for 5 h, then filtered and evaporated in vacuum, separated on silica gel column chromatograph with eluent ethyl acetate/petroleum ether (3:1, v/v) to give the intermediate 1(bromo-ethyl-1*H*-benzo[*c*,*d*]indol-2-one) (0.45 g, yield 81%). Then the intermediate (0.82 g, 3 mmol) was added to the dimethylamine solution (30mL) and kept refluxing for 10 h, extracted with methylene chloride, the oil layer was gathered and evaporated in vacuum, separated on silica gel column chromatograph using eluent ethyl acetate/petroleum ether (1:1, v/v) to give the yellow solid product **A1** 0.54 g, 54% yield. Mp 134–135 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.35 (s, 6H, CH_3N), 2.69 (t, J = 8.02 Hz 2H, CH_2N $(CH_3)_2$), 4.05 (t, J = 7.06 Hz, 2H, CH_2NCO), 6.95 (d, J = 6.93 Hz, 1H, 6-H), 7.46 (t, J = 7.21 Hz, 1H, 7-H), 7.52 (d, J = 8.43 Hz, 1H, 8-H), 7.69 (t, J = 7.13 Hz, 1H, 2-H), 7.99 (d, J = 8.10 Hz, 1H, 1-H), 8.05 (d, J = 6.96 Hz, 1H, 3-H). IR (KBr): 2957, 2890, 1684, 1625, 1470, 770 cm^{-1}; HRMS: $C_{15}H_{16}N_2O$ calculated: 240.1263; found: 240.1263.

4.3.2. 1-[2-(2-Dimethylamino-ethylamino)-ethyl]-1*H*-benzo[c,d]indol-2-one (A3). *N*,*N*-Dimethylethylenediamine (0.31 g, 3.6 mmol) and K_2CO_3 (0.1 g) was added to 1(bromo-ethyl-1*H*-benzo[*c*,*d*]indol-2-one) (0.82 g, 3 mmol) in acetonitrile 30 ml, the mixture was stirred and refluxed for 5 h, then filtered and evaporated, separated on silica gel with eluent ethyl acetate/$CHCl_3$ (5:1, v/v), yellow solid product **A3** was obtained 0.58 g, yield 68%. Mp 96–97 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.19 (s, 6H, CH_3N), 2.41 (t, J = 8.4 Hz, 2H, CH_2N CH_3), 2.76 (t, J = 8.0 Hz, 2H, CH_2NH), 3.03 (t, J = 8.8 Hz, 2H, CH_2CH_2NCO), 4.05 (t, J = 9.2 Hz, 2H, CH_2NCO), 6.97 (d, J = 9.2 Hz, 1H, 6-H), 7.45 (d, J = 9.2 Hz, 1H, 8-H), 7.51 (t, J = 11.2 Hz, 1H, 7-H), 7.69 (t, J = 9.6 Hz, 1H, 2-H), 8.00 (d, J = 8.4 Hz, 1H, 1-H), 8.05 (d, J = 9.6 Hz, 1H, 3-H). IR (KBr): 3310, 2925, 2920, 1689, 1601, 756 cm^{-1}; HRMS: $C_{17}H_{21}N_3O$ calculated: 283.1685; found: 283.1683.

4.3.3. 1-(3-Dimethylamino-propyl)-1*H*-benzo[*c*,*d*]indol-2-one (A2). 1*H*-Benzo[*c*,*d*]indol-2-one (1.69 g, 0.01 mol), 1,3-dichloropropane (2 g, 0.012 mol) and K_2CO_3 (0.05 g) were refluxed in acetonitrile (50 ml) for 5 h, then filtered and evaporated in vacuum, separated on silica gel column chromatograph with eluent ethyl acetate/petroleum (3:1, v/v) to give 2(chloro-propyl-1*H*-benzo[*c*,*d*]indol-2-one) 1.3 g, yield 81%. **2** (0.82 g, 3 mmol) was then added to the dimethylamine solution (2 mmol) and kept refluxing for 10 h, extracted with methylene chloride, the oil layer was gathered and evaporated in vacuum, separated on silica gel column chromatograph with ethyl acetate/petroleum ether = 1:1, v/v) to give yellow solid product **A2** 0.22 g, yield 44%, Mp 122–123 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.26 (t, J = 6.91 Hz, 2H, 2-H, CH_2CH_2N), 2.64 (s, 6H, CH_3N), 3.03 (s, 2H, CH_2NCH_3), 4.09 (t, J = 6.92 Hz, 2H, CH_2NCO), 7.32 (d, J = 7.05 Hz, 1H, 6-H), 7.55 (t, J = 7.16 Hz, 1H, 7-H), 7.62 (d, J = 8.43 Hz, 1H, 8-H), 7.81 (t, J = 7.16 Hz, 1H, 2-H), 8.02 (d, J = 6.92 Hz, 1H, 1-H), 8.15 (d, J = 8.11 Hz, 1H, 3-H); IR (KBr): 2949, 2855, 1690, 1470, 1330, 780 cm^{-1}; HRMS: $C_{16}H_{18}N_2O$ calculated: 254.1419; found: 254.1419.

4.3.4. 2-(2-Chloro-ethyl)-6-nitro-2*H*-acenaphthylen-1-one (A4). 1*H*-Benzo[*c*,*d*]indol-2-one (4.0 g, 0.025 mol) was stirred in concentrated sulfuric acid (20 ml, 98%) at 0–5 °C. The mixed acids (HNO_3 1.73 ml 0.025 mol, d = 1.4, 6.5%) in concentrated sulfuric acid (5 ml) was

added drop-wise, keeping the temperature below 20 °C, then stirred at room temperature for 1.5 h. The reactant was then poured into water (400 ml), filtered, and washed with water to get **3** (4-nitro-1*H*-benzo[*c*,*d*]indol-2-one) 4.5 g, 85% yield; **3** (0.85 g, 4 mmol) was refluxed with 1,2-dichloroethane 5 ml (0.06 mol) and K_2CO_3 (0.1 g) in acetonitrile (30 ml) for 2 h, cooled, removed the solvent, and separated on silica gel chromatograph with ethyl acetate/petroleum ether (1:1, v/v) to afford the yellow needle product **A4** 0.7 g, yield 65%. Mp 217–218 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 4.03 (t, J = 6.11 Hz, 2H, CH_2NCO), 4.4 (t, J = 6.12 Hz, 2H, CH_2Cl), 7.44 (d, J = 8.09 Hz, 1H, 5-H), 8.10 (td, J_1 = 7.13 Hz, J_2 = 1.31 Hz, 1H, 6-H), 8.22 (d, J = 6.99 Hz, 1H, 7-H), 8.69 (d, J = 8.09 Hz, 1H, 2-H), 8.97 (d, J = 8.50 Hz, 1H, 1-H), IR (KBr): 3093, 2910, 1731, 1513, 1326, 749 cm^{-1}; HRMS: $C_{13}H_9N_2O_3$ ^{35}Cl calculated: 276.0302; found: 276.0294; $C_{13}H_9N_2O_3$ ^{37}Cl calculated: 278.0272; found: 278.0284.

4.3.5. Di-1*H*-benzo[*c*,*d*]indol-2-one-ethyl-amine (A5). Diethanolamine (15.75 g, 0.14 mol) in $CHCl_3$ 20 ml was added to $SOCl_2$ in $CHCl_3$ 30 ml drop-wise at 0–5 °C, then stirred at room temperature for 3 h, the excessive $SOCl_2$ was evaporated in vacuum to give the white solid *N*,*N*-dichloroethylamine, 17.5 g, 82% yield. 1*H*-Benzo [*c*,*d*]indol-2-one (2.0 g, 0.01 mol), *N*,*N*-dichloroethylamine (0.6 g, 0.004 mol), and K_2CO_3 (0.1 g) were stirred and refluxed in DMF 30 ml for 10 h, the mixture was then poured into ice-water to precipitate yellow solid, filtered, and separated on silica gel column chromatograph with eluent ethyl acetate/ethanol/chloroform (1:0.4:0.4, v/v/v) to give pale yellow product **A5** 0.8 g, yield 50%. Mp 144–145 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.05 (t, J = 6.36 Hz, 2H, CH_2NH), 3.99 (t, J = 6.33 Hz, 2H, CH_2NCO) 7.09 (d, J = 7.09 Hz, 1H, 6-H), 7.43 (t, J = 8.39 Hz, 1H, 7-H), 7.54 (d, J = 8.44 Hz, 1H, 8-H), 7.77 (t, J = 7.76 Hz, 1H, 2-H), 7.96 (d, J = 6.91 Hz, 1H, 1-H), 8.10 (d, J = 8.09 Hz, 1H, 3-H), IR (KBr): 3307, 2925, 2851, 1696, 1625, 773 cm^{-1}; HRMS: $C_{26}H_{21}N_3O_2$ calculated: 408.1712 (M+1), 409.1746 (M+2), 430.1531 (M+Na); found: 408.1703 (M+1), 409.1774 (M+2), 430.1558 (M+Na).

4.4. Cytotoxicity in vitro evaluation

The cytotoxicities of the synthesized compounds were evaluated by The National Center for Drug Screening.

Acknowledgments

The National Basic Research Program of China (2003CB114400), National Natural Science Foundation of China, and The Science and Technology Foundation of Shanghai supported this study.

References and notes

1. Zhi-Fu, Tao.; Nan-Horng, Lin. *Anti-cancer Agent Med. Chem.* **2006**, *6*, 1–12.
2. Hurley, L. H. *Nature* **2002**, *2*, 188–200.
3. Bourdouxhe-Housiaux, C.; Colson, P.; Houssier, C.; Waring, M. J.; Bailly, C. *Biochemistry* **1996**, *35*, 14.
4. Brana, M. F.; Cacho, M.; Gradillas, A.; de Pascual-Teresa, B.; Ramos, A. *Curr. Pharm. Des.* **2001**, *7*, 17.
5. Bailly, C.; He'nichart, J. P. *Bioconjugate Chem.* **1991**, *2*, 379–393.
6. (a) Brana, M. F.; Castellano, J. M.; Rolda'n, C. M.; Santos, A.; Va'zquez, D.; Jime'nez, A. *Cancer Chemother. Pharmacol.* **1980**, *4*, 61–66; (b) Brana, M. F.; Sanz, A. M.; Castellano, J. M.; Rolda'n, C. M.; Rolda'n, C. *Eur. J. Med. Chem.* **1981**, *16*, 207–212.
7. (a) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. *J. Med. Chem.* **1987**, *30*, 664–669; (b) Baguley, B. C.; Zhuang, L.; Marshall, E. M. *Pharmacology* **1995**, *36*, 244–248.
8. Varney, M. D.; Marzoni, G. P.; Palmer, C. L.; Deal, J. G.; Webber, S.; Welsh, K. M.; Bacquet, R. J.; Bartlett, C. A.; Morse, C. A.; Booth, C. L. J.; Herrmann, S. M.; Howland, E. F.; Ward, R. W.; White, J. *J. Med. Chem.* **1992**, *35*, 663–676.
9. Varney, M. D.; Marzoni, G. P.; Palmer, C. L., et al. *J. Med. Chem.* **1993**, *36*, 1194–1202, and etc.
10. Appelt, K.; Bacquet, R. J.; Bartlett, C. A. *J. Med. Chem.* **1991**, *34*, 1925.
11. (a) Brana, M. F.; Cacho, M.; Gradillas, A.; Pascual-Teresa, B.; Ramos, A. *Curr. Pharm. Des.* **2001**, *7*, 1745–1780; (b) Brana, M. F.; Ramos, A. *Curr. Med. Chem: Anti-cancer Agents* **2001**, *1*, 237–255; (c) Qian, X.; Huang, T., et al. *J. Chem. Soc., Perkin. Trans. 2* **2000**, 715–718; (d) Li, Z.; Yang, Q.; Qian, X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3143–3146.
12. (a) Heby, O.; Persson, L. *TIBS* **1990**, *15*, 153; (b) Goldemberg, S. H.; Algranati, I. D., Eds; *The Biology and Chemistry of Polyamines*, ICSU Symposium; Series; 1989, Vol. 12.
13. Cullis, P. M.; Symmons, M. C. R.; Martyn, C. R.; Cohen, G. M.; Wardman, P. A. *Med. Sci. Res.* **1990**, *18*, 87–88.
14. Marquet, R.; Houssier, C. *J. Biomol. Struct. Dyn.* **1988**, *6*, 235.
15. Marquet, R.; Houssier, C.; Fredericq, E. *Biochim. Biophys. Acta* **1985**, *825*, 365.
16. (a) Rodger, Alison; Taylor, S.; Adlam, G.; Blagbrough, I. S.; Haworth, I. S. *Bioorg. Med. Chem.* **1995**, *3*, 861–872; (b) Atwell, G. J.; Cain, B. F.; Denny, W. A. *J. Med. Chem.* **1977**, *20*, 1128–1134; (c) Ghaneolhosseini, H.; Tjarks, W.; Sjoberg, S. *Tetrahedron* **1998**, *54*, 3877–3884.
17. Kerwin, S. M.; Chen, G.; Kern, J. T.; Thomas, P. W. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 447–450.
18. (a) Xi, Z.; Jones, G. B.; Qabaja, G.; Wright, J.; Johnson, F.; Goldberg, I. H. *Org. Lett.* **1999**, *9*, 1375–1377; (b) Chen, W.-H.; Qin, Y.; Cai, Z.; Chi; Chan, L.; Luod, G.-A.; Jiang, Z.-H. *Bioorg. Med. Chem.* **2005**, *13*, 1859–1866.
19. Geall, A. J.; Blagbrough, I. S. *Tetrahedron* **2000**, *56*, 2449–2460.
20. Gershon, H.; Ghirlando, R.; Guttman, S. B.; Minsky, A. *Biochemistry* **1993**, *32*, 7143.
21. Yang, Q.; Xu, J.; Sun, Y.; Li, Z.; Li, Y.; Qian, X. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 803–806.