Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 11895–11901

Novel DNA bis-intercalators of isoquinolino[4,5-*bc*]acridines: design, synthesis and evaluation of cytotoxic activity

Peng Yang,[a] Qing Yang[b] and Xuhong Qian[a,c,*]

[a]*State Key Laboratory of Fine Chemicals, Dalian University of Technology, PO Box 158, Dalian 116012, China*
[b]*Department of Bioscience and Biotechnology, Dalian University of Technology, Dalian 116012, China*
[c]*Shanghai Key Laboratory of Chemical Biology, East China University of Science and Technology, Shanghai 200237, China*

Received 27 June 2005; revised 12 September 2005; accepted 16 September 2005

Available online 19 October 2005

Abstract—Mono- and dinuclear isoquinolino[4,5-*bc*]acridine derivatives were designed and facilely synthesized, their DNA-binding affinities and cytotoxic activities were evaluated. **A4** induced unwinding of supercoiled plasmid pBR 322 DNA by (36±2)° while **A6** induced that by (41±1)°, both of which were higher than the mono-analogue **A1** ((19±2)°). **A6** exhibited the highest in vitro antitumor activity against human lung cancer cell (A549) and **A4** was the most active one against murine leukemia cell (P388). DNA binding constant and molecular model indicated that both the length of linker chain and the distance of interchromophore were key impact factors for DNA binding affinity and biological activity.

1. Introduction

DNA intercalators have received much attention due to their therapeutic potential in anticancer treatment.[1] The recent molecular design of novel antitumor agents is focused on bisintercalating compounds, which present higher DNA binding affinities and slower dissociation rates than the corresponding monomers.[2] Over the past two decades, a great number of dimeric forms of DNA intercalators, such as bis-acridinecarboxamides, bis-naphthalimides, and bis-imidazoacridones (see Fig. 1), have been developed as potential anticancer drugs.[3]

DNA intercalators usually exhibit a planar structure with at least two annelated aromatic rings, also termed as chromophores, and have one or two flexible basic side chains such as polyamides. Many of these compounds were reported to function as DNA-targeted topoisomerase I and/or II inhibitors.[4] Both naphthalimide and acridine derivatives have proved valuable chromophores in anti-tumor activity and various modifications have been attempted to promote their bioactivities.[4]

The heterocyclic-fused larger ring system, in particular in dimeric forms, was found to account for the high DNA affinity and antitumor activity. As we know well, bis-heterocycle modified bis-naphthalimide and bis-acridine derivatives have been extensively studied for their cytotoxicity, antitumor, and antiparasitic activities.[5,6] However, the reports that could take full advantage of the structural characteristics of both naphthalimide and acridine chromophores seldom appeared. In our continuous attempt[7] to develop high DNA binding affinity and highly active antitumor agents, we designed and synthesized novel mono- and bis-isoquinolino[4,5-*bc*]acridine derivatives **A1**–**A6** (Figure 2). In this study, naphathalimide active group was

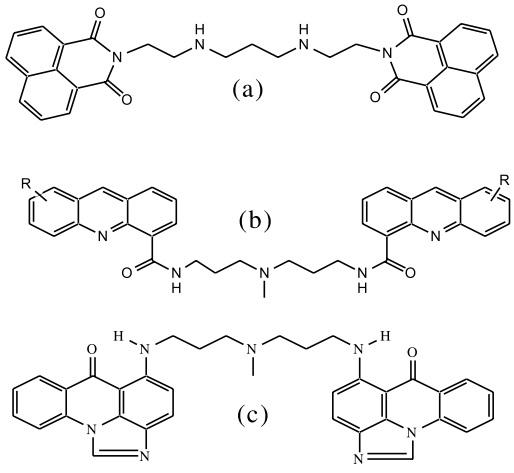
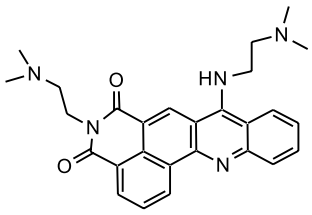


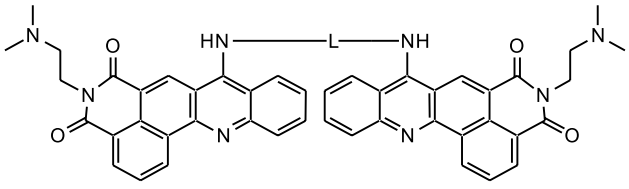
Figure 1. Structure of bis-naphthalimides (a), bis-acridinecarboxamides (b), and bis-imidazoacridones (c).

Keywords: Acridine; DNA-intercalation; Cytotoxicity.
* Corresponding author. Tel.: +86 411 83673466; fax: +86 411 83673488; e-mail addresses: xhqian@dlut.edu.cn; xhqian@ecust.edu.cn

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.09.065



A1



A2 L= $(CH_2)_2NH(CH_2)_2$
A3 L=$(CH_2)_3NH(CH_2)_3$
A4 L=$(CH_2)_3NCH_3(CH_2)_3$
A5 L=$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$
A6 L=$(CH_2)_2NH(CH_2)_3NH(CH_2)_2$

Figure 2. The structures of mono- and dinuclear isoquinolino[4,5-*bc*]acridine derivatives **A1**–**A6**.

remained and the acridine unit was effectively fused with electron-deficiency group. Also, several polyamine chains ranging from 7.3–12.3 Å were used as linkers to bridge two heterocyclic-fused acridine chromophores at the 8-site of these compounds.[8] This resulted structure therefore distinguished our work from the common bisintercalating mode of naphthalimide derivatives and was expected to show higher DNA intercalative property and better antitumor activity due to the presence of two intercalator groups, naphthalimide and acridine.

2. Results and discussion

2.1. Synthesis and spectra

The facile syntheses of these compounds were outlined in Scheme 1. To avoid the imidation of naphthalic anhydride by 2-aminobenzoic acid, 4-bromo-1, 8-naphthalic anhydride, the starting material, was reacted with *N*,*N*-dimethylethylenediamine in ethanol for 2 h as the initial reaction step. Then, the naphthalimide derivative **1** was coupled with 2-aminobenzoic acid in DMF, catalyzed by CuI and Cu.[9] The obtained intermediate **2** was not purified and directly carried out the ring closure reaction in phosphorus oxychloride.[10] The ring closure was proceeded through the formation of a mixed anhydride followed by intramolecular acylation and elimination of hydrochloric acid.[10] And then, it was poured into the ice-cold aqueous solution of NH_4OH, filtered, dried, and separated on silica gel chromatography ($CHCl_3$/MeOH = 9:1, v/v), to give yellow product **3**. Mention should be made here that this ring closure reaction could reach satisfactory yields (88%) of the desired product. Since the compound **3** was of the characteristics of the strong electron-deficiency, the substituent chloride would be a good leaving group, which made the compounds **A2**–**A6** easily obtained by the reaction with different polyamines and the yields were acceptable (from 90 to 33%). However, polyamines were highly hygroscopic and could adopt a different salt, which made elementary analysis an inadequate method of measuring the purity of these compounds.[11] Thus, structures of the obtained novel mono- and bisintercalators were confirmed by IR, ^{1}H NMR and H RMS after purification by silica gel chromatography ($CHCl_3$, MeOH, and NH_4OH). And the UV–vis and fluorescent spectra for these compounds were measured and the data were shown in Table 1.

The linkers of **A2**–**A6** were designed to enable the bis-acridine chromophores to extend over a distance of 7.3–12.3 Å and thus could form the bisintercalated DNA complex.[8]

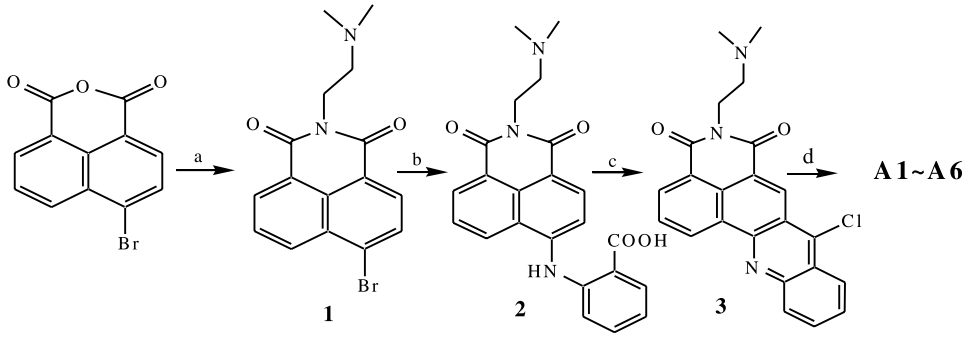
2.2. DNA unwinding property

To illustrate the intercalating mode by the bis-acridine derivatives, we investigated their unwinding ability of supercoiled closed circular DNA pBR322 by electrophoretic mobility measurements on 2% agarose gels. Figure 3 showed the difference of electrophoretic mobility between the intact supercoiling pBR322 and the treated pBR322 DNA by **A1**, **A4**, and **A6** in the range of 0.005–0.090 molar ratios of intercalator per nucleotide (r). The DNA unwinding angle was calculated from the Eq. (1):[12]

$$\phi = 18\ \sigma/r(c) \quad (1)$$

where σ is the superhelical density, $r(c)$ is the molar ratio of intercalator per nucleotide at the coalescence point.

In Figure 3, the DNA unwinding angles for compounds **A1**, **A4**, and **A6** were (19±2)°, (36±2)°, (41±1)°, respectively.



Scheme 1. Synthesis of mono- and bis-isoquinolino[4,5-*bc*]acridines derivatives (a) *N*,*N*-dimethylethylenediamine, ethanol, reflux, 2 h, 85% yield; (b) 2-aminobenzoic acid, Cu/CuI, DMF, 100 °C, 24 h, 86% yield; (c) phosphorus oxychloride, 110 °C,12 h, 88% yield; (d) corresponding polyamine, acetonitrile, reflux, 10 h, 90–33% yield.

Table 1. Spectra data[a,b], properties of the linkers and cytotoxicity (A-549,[c] P388[d]) of compounds

Compounds	UV λ_{max} (nm) (log ε)	FL λ_{max} (nm) (Φ)	Interchromophore		Cytotoxicity [IC_{50} (μM)]	
			Linker	Distance (Å)	A549	P388
A1	462(4.04)	544(0.056)	NA[e]	NA	3.71	0.275
A2	472(3.94)	558(0.024)	$(CH_2)_2NH(CH_2)_2$	7.3	56.2	122
A3	473(4.19)	550(0.015)	$(CH_2)_3NH(CH_2)_3$	9.8	0.150	0.472
A4	473(4.20)	567(0.010)	$(CH_2)_3NCH_3(CH_2)_3$	9.8	0.333	0.246
A5	474(4.27)	557(0.024)	$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$	11.0	0.547	1.03
A6	470(3.88)	562(0.012)	$(CH_2)_2NH(CH_2)_3NH(CH_2)_2$	12.3	0.025	0.281

[a] In absolute ethanol.
[b] With rhodamine B in ethanol as quantum yield standard (ϕ=0.97).
[c] Cytotoxicity (CTX) against human lung cancer cell (A549) was measured by sulforhodamine B dye-staining method.
[d] CTX against murine leukemia cells (P388) was measured by microculture tetrazolium–formazan method.
[e] NA, not applicable.

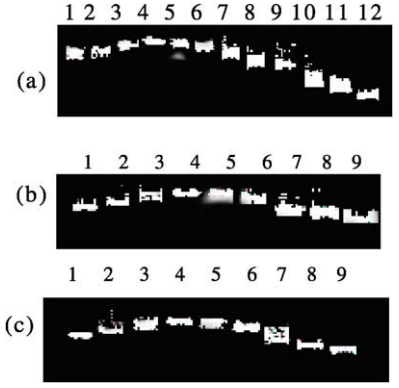


Figure 3. Effects of **A1**, **A4**, and **A6** on the supercoiled pBR 322 DNA. Panel (a) Drug to DNA ratios (*r*) in lanes 1–12 for **A1** are 0.090, 0.085, 0.079, 0.075, 0.072, 0.065, 0.055, 0.04, 0.025, 0.01, 0.005, 0; Panel (b) Drug to DNA ratios (*r*) in lanes 1–9 for **A4**; 0.060, 0.050, 0.043, 0.040, 0.037, 003, 0.02, 0.01, 0; Panel (c) Drug to DNA ratios (*r*) in lanes 1–9 for **A6**: 0.050, 0.045, 0.039, 0.035, 0.033, 0.025, 0.02, 0.01, 0.

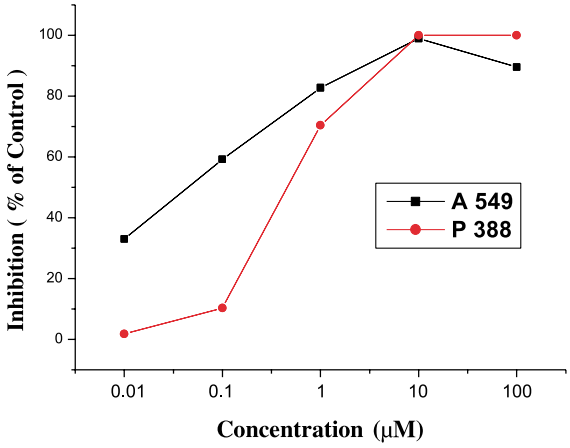


Figure 4. The dose–response curve of the compound **A6** for both A549 and P388.

For **A4** and **A6**, the unwinding angles were 1.9–2.2-fold greater than that of the monomer, 8-(dimethylamino)ethylamino-5-(2-(dimethylamino)ethyl)-5*H*-isoquinolino[4,5-*bc*]acridine-4,6-dione, indicating that **A4** and **A6** may have bis-intercalated with pBR 322 DNA. In addition, the calculated helix-unwinding angles also revealed that linkers played a key role on unwinding supercoiling of DNA.

2.3. Structure–activity relationships for cellular growth inhibition

SRB (Sulforhodamine B) assay against A549 (human lung cancer cell) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against P388 (murine leukemia cell) were used to evaluate the antitumor activities of these compounds. IC_{50} represents the drug concentration (micromolar) required to inhibit cell growth by 50%. The IC_{50} values were calculated based on the parameters in Table 2. Figure 4 presented the dose–response curve of the representative compound **A6** for both A549 and P388. The structure parameters of these compounds and their cytotoxicities (as IC_{50} values) were listed in Table 1. It was showed that these polyamine systems except **A2** were active in the described panels, although their activities were in every case different. The dinuclear NH substituted compound **A6** was found to be the most effective antitumor agent against A549 (IC_{50}, 0.025 μM) and **A4** was more cytotoxic against P388 (IC_{50}, 0.246 μM). However, **A1**, the mononuclear analogue exhibited higher activity towards P388 (IC_{50}, 0.275 μM) than against A549 (IC_{50}, 3.71 μM).

For A549, the dimeric forms of isoquinolino[4,5-*bc*]acridine exhibited 10–20 fold more active cytotoxicity than its

Table 2. Cytotoxic activities of **A1**–**A6** against A549 and P388 at different concentrations (mol/L)

Compounds (M)	Inhibition of tumor growth (%) A549					Inhibition of tumor growth (%) P388				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
A1	98.4	97.2	38.4	0	0	100.0	100.0	39.7	10.7	4.2
A2	50.7	13.8	4.2	3.4	0	30.8	8.9	5.1	2.1	0
A3	84.3	97.8	80.2	39.9	13.8	100.0	100.0	39.5	9.0	0.4
A4	99.6	99.0	43.9	11.2	8.5	100.0	100.0	91.6	10.5	1.2
A5	94.3	82.2	73.8	15.2	12.2	100.0	80.9	6.1	5.4	5.2
A6	89.5	98.9	82.7	59.2	33.0	100.0	100.0	70.4	10.3	1.8

mono-derivative. Compound **A4**, the *N*-methylation of the potent cytotoxic agent **A3**, resulted in approximately a 2-fold less activity. **A5**, with shorter CH_2 spacer between two N atoms, led to nearly 22-fold loss of cytotoxic potency than that of **A6**. Evaluation of the polyamine bridge indicated that **A2**–**A5** did not exhibit good cytotoxic activity as **A6** (12.3 Å) did, which may be caused by their relatively shorter linker (from 7.3 to 11.0 Å) not allowing both intercalator moieties intercalating DNA at the same time. The aminoalkyl linker chain [$(CH_2)_2$–NH–$(CH_2)_3$–NH–$(CH_2)_2$] of **A6**, exhibited decent antitumor activity against both A549 and P388.

There was one interesting trend listed in Table 1 that deserved further discussion. We could see that for both A549 and P388, the alkyl chain between the aminicimidic nitrogens was propylene (**A6**) rather than ethylene (**A5**) exerted greater activity (22-fold and 5-fold, respectively). In order to analyze the difference between **A5** and **A6**, their Scatchard binding constants to calf thymus DNA (in 20 mM Tris–HCl buffer, pH 7.0) were determined based on the method of fluorescence quenching technique.[13] Figure 5 presented the Scatchard plots of spectrophotometric titrations of CT-DNA to the representative compound **A6**. The calculated Scatchard binding constants for **A5** and **A6** were $3.56\times10^4\ M^{-1}$ and $2.37\times10^5\ M^{-1}$, respectively, indicating that **A6** could form a more stable DNA complex. The order of Scatchard constants and the binding site size were similar to those in the reference.[14a–c]

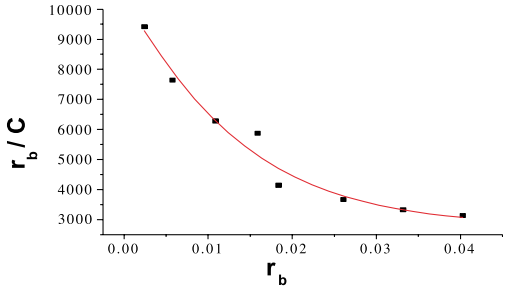


Figure 5. Scatchard plots of spectrophotometric titration of CT-DNA to **A6** in Tris–HCl buffer. A plot of r_b/c versus r_b gives the association constant (slope, $2.37\times10^5\ M^{-1}$) and the apparent number of binding sites per nucleotide (*x*-intercept, 0.04) for the agent.

To further illustrate the difference between **A5** and **A6** DNA complexes, hyperchem 7.0 package was used to build a simple molecular model. The properly modified AMBER method was selected in this study. The intercalation energies (ΔE) were calculated using $\Delta E = E_{complex} - [E_{compd} + E_{DNA}]$, where $E_{complex}$, E_{compd}, and E_{DNA} were the computed potential energies for the minimized average **A5**/**A6**-DNA complex, free **A5**/**A6** and free DNA.[15]

The images of **A5** and **A6** DNA complexes obtained by molecular modeling were presented in Figure 6. The calculated intercalation energies (ΔE) for **A5** and **A6** were −2.91 and −23.78 kcal/mol, respectively. According to the stable geometries of the bisintercalation complexes and the calculated intercalation energies (ΔE), we concluded that **A6** could exhibit higher DNA affinity than **A5**, which could be caused by the sufficient long interchromophore distance (12.3 Å).[16]

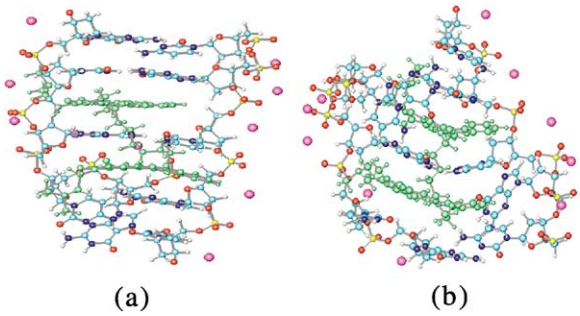


Figure 6. Images of **A5** and **A6** intercalated into d (CGCGC). Carbon atoms are colored in green, nitrogens in blue, oxygens in red and hydrogens in white, phosphorus in yellow and sodium counterions is in pink. All the atoms of **A5** and **A6** are colored in green.

3. Conclusion

The design, facile synthesis, DNA-binding affinity and the cytotoxic activity of novel mono- and dinuclear isoquinolino[4,5-*bc*]acridine derivatives **A1**–**A6** were demonstrated. **A6** exhibited the highest in vitro antitumoral activity against human lung cancer cell (A549) and **A4** was the most active against murine leukemia cell (P388). DNA binding study and molecular modeling of the **A5**/**A6** DNA complexes indicated that **A6** with the long enough linker could exhibit the higher DNA affinity than **A5**, which contributed to its higher antitumor activity.

4. Experimental

4.1. Materials

All the solvents were of analytic grade. ^{1}H NMR was measured on a Bruker AV-400 spectrometer with chemical shifts reported as parts per million (in 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 uncorrected. Absorption spectra were determined on PGENERAL TU-1901 UV–vis spectrophotometer.

4.2. Synthesis

4.2.1. 8-(Dimethylamino)ethylamino-5-(2-(dimethylamino)ethyl)-5*H*-isoquinolino[4,5-*bc*]acridine-4,6-dione (A1):. 4-Bromo-1, 8-naphthalic anhydride (2.77 g, 10 mmol) and *N*,*N*-dimethylethylenediamine (1.0 g, 11.4 mmol) were added to 10 mL ethanol, the reaction mixture was stirred at reflux temperature for 2 h, then cooled, filtered, and dried, the crude product was obtained as yellow solid **1** (2.95 g, 8.5 mmol). APCI-MS (positive) *m*/*z*: 348.2 ($[M+H]^+$). (b) The obtained 4-bromo-1,8-naphthalimide derivative (2 g, 5.76 mmol) and 2-aminobenzoic acid (0.8 g, 6 mmol), copper bronze (0.038 g, 0.6 mmol), CuI (0.101 g, 0.53 mmol), were added to 10 mL DMF. The reaction mixture was stirred at 100 °C for 24 h, filtered while it was hot, and the filtrate was cooled and poured into the ice water, filtered,

and dried to get the red product **2**. (2.0 g, 4.95 mmol, 86% yield) APCI-MS (positive) *m/z*: 404.3 ($[M+H]^+$). This product was not purified and used directly in the next step. (c) 1 g of **2** was stirred in phosphorus oxychloride at 110 °C for 12 h, then cooled and poured into the ice water, $N(Et)_3$ was added when stirred vigorously, filtered, and dried to give yellow solid **3** (0.88 g, 88% yield).

Purified by silica gel chromatography ($CHCl_3$/MeOH=9:1, v/v) to get pure product **3**, Mp: 204.8–205.2 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.99 (s, 6H, NCH_3), 3.51 (s, 2H, NCH_2), 4.69–4.72 (t, J_1=6.0 Hz, J_2=6.0 Hz, 2H, $CONCH_2$), 7.77–7.81 (t, J_1=7.6 Hz, J_2=7.6 Hz, 1H), 7.97–8.04 (m, 2H), 8.39–8.42 (d, J_1=8.8 Hz, 1H), 8.52–8.54 (d, J=8.4 Hz, 1H), 8.72–8.74 (d, J=7.6 Hz, 1H), 9.52 (s, 1H), 9.77–9.79 (d, J=8.0 Hz, 1H), ESI-HRMS: calcd for $C_{23}H_{19}ClN_3O_2$ ($M+H^+$): 404.1166, Found: 404.1166, Found: 404.1158. IR (KBr): 2923, 2853, 1702, 1660, 1347 cm^{-1}.

The above obtained compound (1 g, 2.48 mmol) and *N*,*N*-dimethylethylenediamine (0.26 g, 2.97 mmol) were added to 10 mL acetonitrile. The solution was refluxed for 5 h, cooled and filtered. Separated by silica gel chromatography ($CHCl_3$/MeOH=5:1, v/v) to get pure **A1** (1.01 g, 90% yield), Mp: 148.9–150.4 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.47 (s, 6H, $N(CH_3)_2$), 2.52 (s, 6H, $N(CH_3)_2$), 2.75–2.78 (t, J_1=6.0 Hz, J_2=5.2 Hz, 2H, CH_2), 2.87 (s, 2H, CH_2), 4.06–4.09 (t, J_1=5.6 Hz, J_2=6.0 Hz, 2H, CH_2), 4.42–4.46 (t, J_1=6.8 Hz, J_2=7.2 Hz, 2H, CH_2), 7.52–7.55 (t, J_1=6.8 Hz, J_2=7.6 Hz, 1H), 7.79–7.88 (m, 2H), 8.13–8.15 (d, J=8.4 Hz, 1H), 8.20–8.22 (d, J=8.4 Hz, 1H), 8.61–8.63 (d, J=7.6 Hz, 1H), 9.35 (s, 1H), 9.63–9.65 (d, J=8.8 Hz, 1H), ESI-HRMS: calcd for $C_{13.5}H_{15.5}N_{2.5}O$ ($M+2H^+/2$): 228.6239, Found: 228.6231. IR (KBr): 3397, 2924, 2854, 1692, 1653, 1347 cm^{-1}.

4.3. Synthesis of dinuclear isoquinolino[4,5-*bc*]acridine derivatives A2–A6

The preparation and purification procedure of **A2**–**A6** was similar to that of **A1**: several polyamine chains were selected instead of *N*,*N*-dimethylethylenediamine.

4.3.1. Compound A2. Purified by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH=1:2:0.03, v/v/v), 69% yield, Mp: 191.3–191.5 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.28 (s, 4H, $2CH_2$), 2.45 (s, 12H, $2N(CH_3)_2$), 3.19 (s, 4H, $2CH_2$), 3.71 (s, 4H, $2CH_2$), 4.02 (s, 4H, $2CH_2$), 7.33 (s, 2H), 7.67–7.71 (m, 4H), 7.78–7.80 (d, J=8.4 Hz, 2H), 8.23–8.25 (d, J=8.0 Hz, 2H), 8.28–8.30 (d, J=8.4 Hz, 2H), 8.96 (s, 2H), 9.08–9.10 (d, J=8.4 Hz, 2H), ESI-HRMS: calcd for $C_{25}H_{24.5}N_{4.5}O_2$ ($M+2H^+/2$): 419.6954, Found: 419.6948. IR (KBr): 3427, 2922, 2853, 1696, 1653, 1346 cm^{-1}.

4.3.2. Compound A3. Purified by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH =1:2:0.03, v/v/v), 61% yield, Mp: 195.9–196.1 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.18 (s, 4H, $2CH_2$), 2.32 (s, 12H, $2N(CH_3)_2$), 2.47 (s, 4H, $2CH_2$), 3.18 (s, 4H, $2CH_2$), 3.85 (s, 4H, $2CH_2$), 4.18 (s, 4H, $2CH_2$), 7.32–7.36 (t, J_1=7.2 Hz, J_2=8.0 Hz, 2H), 7.62–7.66 (m, 4H), 7.77–7.79 (d, J=8.4 Hz, 2H), 8.02 (s, br, 2H), 8.32–8.34 (d, J=8.0 Hz, 2H), 8.86 (s, 2H), 8.97–8.99 (d, J=8.0 Hz, 2H), ESI-HRMS: calcd for $C_{17.3}H_{18}N_3O_{1.3}$ ($M+3H^+/3$): 289.4766, Found: 289.4767. IR (KBr): 3399, 2924, 2853, 1689, 1650, 1376 cm^{-1}.

4.3.3. Compound A4. Purified by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH =1:2:0.03, v/v/v), 85% yield, Mp: 213.6–213.8 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.15 (s, 4H, $2CH_2$), 2.30 (s, 4H, $2CH_2$), 2.32 (s, 3H, NCH_3), 2.42 (s, 12H, $2NCH_3$), 2.74 (s, 4H, $2CH_2$), 3.66 (s, 4H, $2CH_2$), 4.01 (s, 4H, $2CH_2$), 7.33–7.37 (t, J_1=7.6 Hz, J_2=7.6 Hz, 2H), 7.33–7.37 (t, J_1=7.6 Hz, J_2=7.6 Hz, 2H), 7.56–7.69 (m, 6H), 8.12–8.14 (d, J=7.2 Hz, 2H), 8.28–8.30 (d, J=8.0 Hz, 2H), 8.71 (s, 2H), 8.85–8.87 (d, J=7.2 Hz, 2H), ESI-HRMS: calcd for $C_{17.7}H_{18.7}N_3O_{1.3}$ ($M+3H^+/3$): 294.1485, Found: 294.1483. IR (KBr): 3431, 2922, 2854, 1691, 1652, 1395 cm^{-1}.

4.3.4. Compound A5. Purified by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH =1:2:0.06, v/v/v), 52% yield, Mp: 159.9–160.1 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.35–2.39 (t, J_1=6.8 Hz, J_2=6.8 Hz, 4H, $2CH_2$), 2.40 (s, 6H, $2NCH_3$), 2.97 (s, 4H, $2CH_2$), 3.13 (s, 4H, $2CH_2$), 3.79 (s, 4H, $2CH_2$), 3.97 (s, 4H, $2CH_2$), 7.33 (s, 2H), 7.57–7.64 (m, 6H), 8.12–8.14 (d, J=6.8 Hz, 2H), 8.29–8.31 (d, J=8.0 Hz, 2H), 8.85 (s, 2H), 8.92–8.94 (d, J=8.4 Hz, 2H), ESI-HRMS: calcd for $C_{26}H_{27}N_5O_2$ ($M+2H^+/2$): 441.2165, Found: 441.2179. IR (KBr): 3419, 2926, 2852, 1693, 1650, 1392 cm^{-1}.

4.3.5. Compound A6. Purified by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH =1:2:0.06, v/v/v), 33% yield, Mp: 102.2–102.5 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.11–2.14 (m, 2H, CH_2), 2.37 (s, 4H, $2CH_2$), 2.54 (s, 12H, $2N(CH_3)_2$), 2.89 (s, 4H, $2CH_2$), 3.03 (s, 4H, $2CH_2$), 3.82 (s, 4H, $2CH_2$), 3.90 (s, 4H, $2CH_2$), 7.40 (s, 2H), 7.60–7.69 (m, 6H), 8.17–8.19 (d, J=7.6 Hz, 2H), 8.26–8.28 (d, J=8.0 Hz, 2H), 8.85 (s, 2H), 8.97–8.99 (d, J=8.0 Hz, 2H), ESI-HRMS: calcd for $C_{26.5}H_{28}N_5O_2$ ($M+2H^+/2$): 448.2243, Found: 448.2260. IR (KBr): 3334, 2924, 2853, 1695, 1654, 1391 cm^{-1}.

4.4. CT-DNA binding studies

The solution of compounds **A5** and **A6** in DMSO (10^{-3}–10^{-4} M) was diluted with 20 mM Tris–HCl (pH 7.0) to the samples at the concentration of 1, 2.5, 5, 7.5, 10, 15, 20, 25 μM, respectively. Then, it was separated to two parts: one contained Calf-thymus DNA 30 μM, the other contained no DNA but the same concentration of chemical as control. All the above solutions were shaken for 3 days at 25 °C in the dark. Fluorescence wavelength and intensity area of samples were measured.

4.5. DNA unwinding angle measurement

Covalently closed circular plasmid DNA, pBR 322, was purchased from TaKaRa Co., Ltd as a 0.5 mg per mL. Prior to application to the agarose gel, pBR322 DNA aliquots were incubated with the compounds at 37 °C in TE buffer (Tris–HCl 10 mm, pH 7.4, EDTA 0.1 mM) for 24 h at several molar ratios of drug to nucleotide. The density of supercoiling was δ −0.08 under our experimental conditions.[17]

The fraction of unreacted drug was separated from the mixture by precipitation of the DNA with 2.5 V of ethanol and 0.3 M sodium acetate, pH 4.8. Two percentage of agarose gel electrophoresis was carried out at 25 V in 40 mM TAE buffer (40 mM tris(hydroxymethyl)aminomethane, 30 mM glacial acetic acid, 1 mM EDTA, pH 7.5). After electrophoresis, the gel was stained with ethidium bromide.

4.6. Molecular modeling methods

The d (CGCGC) was selected as the intercalation site sequence, a preferential feature for cytostatic active principles[17b,18a,b] and the similar intercalative mode by **A5** and **A6** was supposed. According to the parameters of reference,[18c–e] AMBER method was properly modified. In all cases, $\xi = 4r$ was used to simulate the solvent effects Na^+ counterions were included, placed at 6 Å distance from each phosphate–oxygen bisector.

4.7. Cytotoxic activity in vitro

The prepared compounds have been submitted to Shanghai Institute of Materia Medica for testing their cytotoxicities in vitro.

A549 (human lung cancer cell) and P388 (murine leukemia cell) were seeded into 96 well cell culture plates before experimental manipulation, and then treated with a test compound each at different concentrations for 72 h. At the end of treatments, A549 and P388 cell numbers were determined by SRB and MTT assay, respectively.[19,20]

For the MTT assay, briefly, a 20 μL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5 mg/mL) was added directly to all the appropriate wells. The cultures were then incubated for 4 h. Then 100 μL of 'triplex solution' (10% SDS/ 5% isobutanol/ 12 mM HCl) was added. After the plates were incubated at 37 °C overnight, they were measured by the absorbance at 570 nm using a multiwell spectrophotometer.

For the SRB assay, in brief, the cells were fixed with 10% trichloroacetic acid for an hour at 4 °C. After they were extensively washed, fixed cells were stained for 30 min with 0.4% sulforhodamine B (SRB) in 1% acetic acid. Unbound SRB was washed away with 1% acetic acid. Then, the cultures were air-dried. Bound dye was solubilized with 10 mM Tris (pH 10.5) prior to reading plates. The OD value was read on a plate reader at a wavelength of 515 nm. The ratio of the OD (optical density) value of a compound-treated culture to the OD value of a mock-treated culture, expressed in percentage, was used to quantify the cytotoxicity of a compound. Results were expressed as IC_{50} (the drug concentration that reduces by 50% the absorbance in treated cells with respect to untreated cells).

The cytotoxic activities of **A1–A6** against the selected tumor cell lines A549 and P388 at different concentrations were listed in Table 2. The numbers 10^{-4}–10^{-8} were the concentrations of the test compounds while the numbers in the matrix were the ratio of the OD of the treated sample relative to that of the control sample. IC_{50} in Table 1 was calculated based on these parameters.

Acknowledgements

Financial support by National Basic Research Program of China(2003CB114400) and under the auspices of 863 plan for High-Tech (2003AA2Z3520), is greatly appreciated. This work is also supported by Dalian Excellent Young Scientist Research Fund.

References and notes

1. Kochevar, E. D. D. In *Dunn in Bioorganic Photochemistry*; Morrison, H., Ed.; Wiley: New York, 1990; pp 273–283.
2. (a) Wakelin, L. P. G. *Med. Res. Rev.* **1986**, *6*, 275–340. (b) Hsiang, Y. H.; Jiang, J. B.; Liu, L. F. *Mol. Pharmacol.* **1989**, *36*, 371–376. (c) Cholody, W. M.; Hernandez, L.; Hassner, L.; Scudiero, D. A.; Djurickovic, D. B.; Michejda, C. J. *J. Med. Chem.* **1995**, *38*, 3043–3052. (d) Spicer, J. A.; Gamage, S. A.; Atwell, G. J.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. *Anti-Cancer Drug Des.* **1999**, *14*, 281–289.
3. Bousquet, P. F.; Brana, M. F.; Conlon, D.; Fitzgerald, K.; Perron, M. D.; Cocchiaro, C.; Miller, R.; Moran, M.; George, J.; Qian, X. D.; Keilhauer, G.; Romerdahl, C. A. *Cancer Res.* **1995**, *55*, 1176–1189.
4. (a) Laursen, J. B.; Nielsen, J. *Chem. Rev.* **2004**, *104*, 1663–1686. (b) Blanchard, S.; Rodriguez, I.; Tardy, C.; Baldeyrou, B.; Bailly, C.; Colson, P.; Houssier, C.; Leonce, S.; Kraus, B. L.; Pfeiffer, B.; Renard, P.; Pierre, A.; Caubere, P.; Guillaumet, G. *J. Med. Chem.* **2004**, *47*, 978–987. (c) Hay, M. P.; Pruijn, F. B.; Gamage, S. A.; Liyanage, H. D. S.; Kovacs, M. S.; Patterson, A. V.; Wilson, W. R.; Brown, J. M.; Denny, W. A. *J. Med. Chem.* **2004**, *47*, 475–488. (d) Antonini, I.; Polucci, P.; Magnano, A.; Gatto, B.; Palumbo, M.; Menta, E.; Pescalli, N.; Martelli, S. *J. Med. Chem.* **2003**, *46*, 3109–3115. (e) Rastogi, K.; Chang, J.-Y.; Pan, W.-Y.; Chen, C.-H.; Chou, T.-C.; Chen, L.-T.; Su, T.-L. *J. Med. Chem.* **2002**, *45*, 4485–4493. (f) Gamage, S. A.; Spicer, J. A.; Rewcastle, G. W.; Milton, J.; Sohal, S.; Dangerfield, W.; Mistry, P.; Vicker, N.; Charlton, P. A.; Denny, W. A. *J. Med. Chem.* **2002**, *45*, 740–743. (g) Wang, S.; Miller, W.; Milton, J.; Nigel, V.; Alistair, S.; Peter, C.; Prakash, M.; David, H.; William, A. D. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 415–418. (h) Brana, M. F.; Ramos, A. *Curr. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 237–255. (i) Rogers, J. E.; Weiss, S. J.; Kelly, L. A. *J. Am. Chem. Soc.* **2000**, *122*, 427–436. (j) Qian, X.; Huang, T. B.; Wei, D.-Z.; Zhu, D. H.; Fan, M. C.; Yao, W. *J. Chem. Soc., Perkin Trans. 2* **2000**, 715–718. (k) Rogers, J. E.; Kelly, L. A. *J. Am. Chem. Soc.* **1999**, *121*, 3854–3861. (l) Brana, M. F.; Castellano, J. M.; Perron, D.; Maher, C.; Conlon, D.; Bousquet, P. F.; George, J.; Qian, X.-D.; Robinson, S. P. *J. Med. Chem.* **1997**, *40*, 449–454.
5. Brana, M. F.; Cacho, M.; Garcia, M. A.; Pascual, T. B.; Ramos, A.; Dominguez, M. T.; Pozuelo, J. M.; Abradelo, C.; Rey, S. M. F.; Yuste, M.; Banez, C. M.; Lacal, J. C. *J. Med. Chem.* **2004**, *47*, 1391–1399.

6. Girault, S.; Grellier, P.; Berecibar, A.; Maes, L.; Mouray, E.; Lemiere, P.; Debreu, M. A.; Davioud, C. E.; Sergheraert, C. *J. Med. Chem.* **2000**, *43*, 2646–2654.
7. (a) Yang, Q.; Qian, X. H.; Xu, J. Q.; Sun, Y. S.; Li, Y. G. *Bioorg. Med. Chem.* **2005**, *13*, 1615–1622. (b) Qian, X. H.; Li, Y. G.; Xu, Y. F.; Liu, D.; Qu, B. Y. *Bioorg. Med. Chem.* **2004**, *14*, 2665–2668. (c) Xu, Y. F.; Qian, X. H.; Yao, W.; Mao, P.; Cui, J. *Bioorg. Med. Chem.* **2003**, *11*, 5427–5433. (d) Qian, X. H.; Yao, W.; Chen, G.; Huang, X. Y.; Mao, P. *Tetrahedron Lett.* **2001**, *42*, 6175–6178.
8. Wakelin, L. P.; Romanos, M.; Chen, T. K.; Glaubiger, D.; Canellakis, E. S.; Waring, M. J. *Biochemistry* **1978**, *17*, 5057–5063.
9. Baudoin, O.; Teulade, F. P. M.; Vigneron, J. P.; Lehn, J. M. *J. Org. Chem.* **1997**, *62*, 5458–5470.
10. Acheson, R. M.; Acridines, the chemistry of heterocyclic compounds; Wiley: London, 1973; 9; pp 563–574.
11. Byk, G.; Dubertret, C.; Escriou, V.; Frederic, M.; Jaslin, G.; Rangara, R.; Pitard, B.; Crouzet, J.; Wils, P.; Schwartz, B.; Scherman, D. *J. Med. Chem.* **1998**, *41*, 224–235.
12. Keck, M. V.; Lippard, S. J. *J. Am. Chem. Soc.* **1992**, *114*, 3386–3390.
13. Gupta, M.; Ali, R. *J. Biochem.* **1984**, *95*, 1253–1257.
14. (a) Wirth, M.; Buchardt, O.; Koch, T.; Nielsen, P. E.; Norden, B. *J. Am. Chem. Soc.* **1988**, *110*, 932–939. (b) Boger, D. L.; Ichikawa, S.; Tse, W. C.; Hedrick, M. P.; Jin, Q. *J. Am. Chem. Soc.* **2001**, *123*, 561–568. (c) Antonini, I.; Polucci, P.; Magnano, A.; Sparapani, S.; Martelli, S. *J. Med. Chem.* **2004**, *47*, 5244–5250.
15. Cairns, D.; Michalitsi, E.; Jenkins, T. C.; Mackay, S. P. *Bioorg. Med. Chem.* **2002**, *10*, 803–807.
16. Bailly, C.; Carrasco, C.; Joubert, A.; Bal, C.; Wattez, N.; Hildebrand, M. P.; Lansiaux, A.; Colson, P.; Houssier, C.; Cacho, M.; Ramos, A.; Brana, M. F. *Biochemistry* **2003**, *42*, 4136–4150.
17. (a) Wakelin, L. P. G.; Bu, X.; Eleftheriou, A.; Parmar, A.; Hayek, C.; Stewart, B. W. *J. Med. Chem.* **2003**, *46*, 5790–5802. (b) Brana, M. F.; Casarrubios, L.; Dominguez, G.; Fernandez, C.; Perez, J. M.; Quiroga, A. G.; Navarro-Ranninger, C.; de Pascual-Teresa, B. *Eur. J. Med. Chem.* **2002**, *37*, 301–313.
18. (a) Ornstein, R. L.; Rein, R. *Biopolymers* **1979**, *18*, 2821–2847. (b) Chaires, J. B. *Biophys. Chem.* **1990**, *35*, 191–202. (c) Marinela, R. C.; Sanz, A. M.; Yunta, M. J. R.; Fernando, G. C.; Navarro, P.; Fernandez, I.; Pardoa, M.; Canoa, C. *Bioorg. Med. Chem.* **2003**, *11*, 2143–2148. (d) Fox, T.; Scanlan, T. S.; Kollman, P. A. *J. Am. Chem. Soc.* **1997**, *119*, 11571–11577. (e) Grootenhuis, P. D.; Kollman, P. A. *J. Am. Chem. Soc.* **1989**, *111*, 2152–2158.
19. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
20. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.