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Spacer length and attaching position-dependent binding of synthesized protoberberine dimers to double-stranded DNA

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Abstract—Six jatrorrhizine homodimers and berberine–jatrorrhizine heterodimers have been synthesized in moderate to good yields from the reaction of jatrorrhizine with α,ω-dibromoalkanes and 9-*O*-(ω-bromoalkyl)berberines, respectively. Their binding activities toward calf thymus (CT) DNA and three double-stranded oligodeoxynucleotides, $d(AAGAATTCTT)_2$, $d(TAAGAATTCTTA)_2$, and $d(TTAAGAATTCTTAA)_2$, were investigated by means of spectrofluorimetric and spectrophotometric titrations. The results indicate that these dimers exhibit enhanced DNA-binding affinities due to the cooperative interaction of the two protoberberine subunits. A comparative study of the DNA-binding behaviors of berberine homodimers, jatrorrhizine homodimers, and berberine–jatrorrhizine heterodimers suggests that spacer length and attaching position are of great importance in modulating their DNA-binding affinities.

1. Introduction

A number of small organic molecules are endowed with attractive biological activities that are exerted through specific and noncovalent interactions with DNA.[1] These interactions disclose the molecular basis of many antitumor and antivirus drugs and provide useful guidance for the rational design of new potential chemotherapeutic agents.[2] Therefore, in recent years there has been an increasing interest in exploiting novel efficient DNA ligands that can target DNA with high binding affinities and site specificities.[3]

In these aspects, considerable attentions have been paid to naturally occurring protoberberine alkaloids. This is because protoberberine alkaloids exhibit extensive pharmacological activities,[4] such as antimicrobial, antileukemic, anticancer, and topoisomerase inhibitory activities. On the other hand, berberine, one of the most studied among the protoberberine alkaloids, has been demonstrated to be capable of forming complex with DNA.[5,6] These two features of berberine have highlighted the potentials of protoberberine alkaloids as a promising lead compound for the development of effective DNA-binders.

The binding strengths of berberine, palmatine, and their analogues, however, are moderate, which necessitates structural modification as to afford strong DNA-binding agents. In previous study, we have reported the synthesis of berberine dimers (i.e., Ber-C_{2-4}-Ber, Scheme 1) and their remarkably enhanced DNA-binding affinities that can be modulated by varying the spacer lengths.[7] To further clarify the structure–activity correlation, we describe herein the facile synthesis of analogous dimers, jatrorrhizine homodimers (Jat-C_{2-4}-Jat) and berberine–jatrorrhizine heterodimers (Ber-C_{2-4}-Jat) (Scheme 1), and a comparative study of their DNA-binding behaviors with those of Ber-C_{2-4}-Ber. These three types of protoberberine dimers are linked in a different way and palmatine has comparable DNA-binding affinity with berberine,[6b,8] thus this comparative study may

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† The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint first authors.

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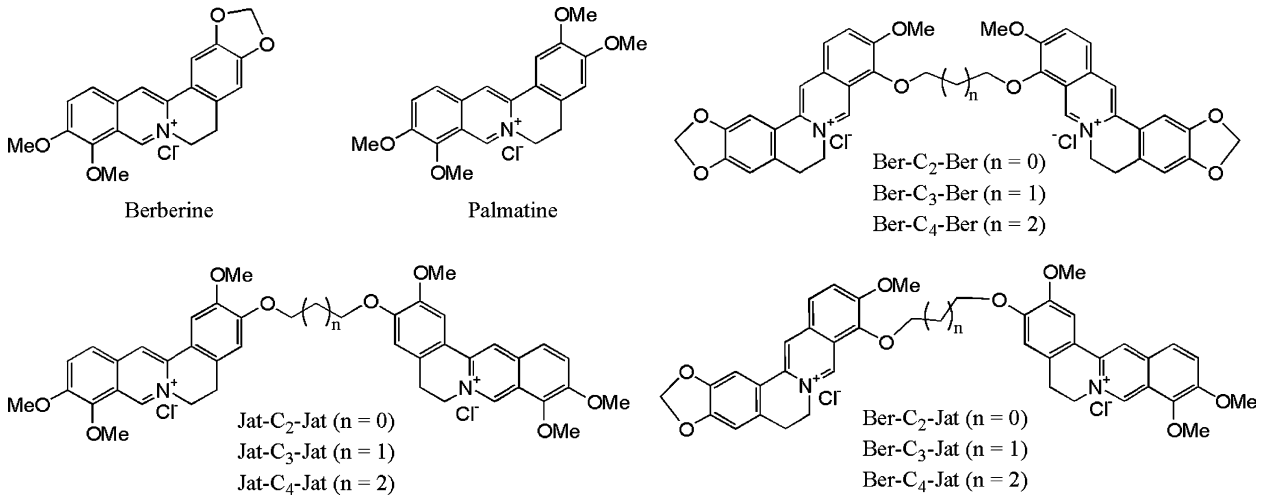


Scheme 1. Structures of berberine, palmatine, berberine homodimers (Ber-C_{2-4}-Ber), jatrorrhizine homodimers (Jat-C_{2-4}-Jat), and berberine–jatrorrhizine heterodimers (Ber-C_{2-4}-Jat).

provide more insight into the structure–activity correlations involved in the DNA-binding processes of protoberberine dimers.

2. Results and discussion

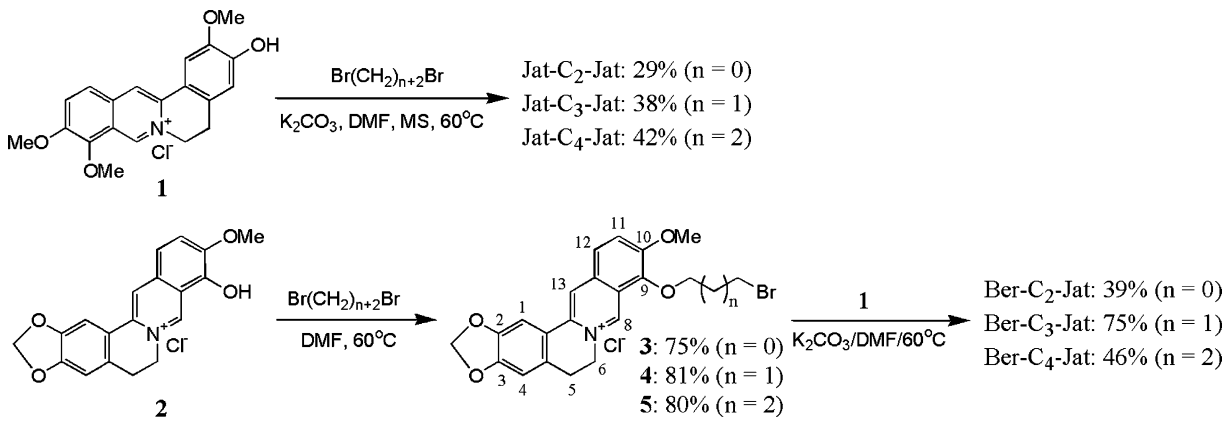
2.1. Synthesis of Jat-C_{2-4}-Jat and Ber-C_{2-4}-Jat

Jat-C_{2-4}-Jat and Ber-C_{2-4}-Jat were synthesized as described in Scheme 2. The coupling reaction of jatrorrhizine (**1**) with α,ω-dibromoalkanes in the presence of K_2CO_3 afforded Jat-C_{2-4}-Jat in 29–42% yields. Ber-C_{2-4}-Jat were synthesized in 39–75% yields from the reaction of **1** with the corresponding 9-*O*-(ω-bromoalkyl)berberines **3**–**5** which were prepared from the alkylation (75–81%) of berberrubine **2**[9] by the corresponding α,ω-dibromoalkanes. These dimers were fully characterized by HR-ESI-MS and NMR (^{1}H and ^{13}C). All these compounds afforded two-charged ESI-MS peaks ($[M-2Cl]^{2+}$) in the mass spectra. Their NMR spectra, for example, ^{1}H NMR of Jat-C_2-Jat in Figure 1, were also consistent with the given structures.

2.2. Interaction of Jat-C_{2-4}-Jat and Ber-C_{2-4}-Jat with DNA

The binding affinities of Jat-C_{2-4}-Jat and Ber-C_{2-4}-Jat toward three self-complementary double-stranded oligodeoxynucleotides, $d(AAGAATTCTT)_2$, $d(TAAGAATTCTTA)_2$, and $d(TTAAGAATTCTTAA)_2$, were evaluated by means of spectrofluorimetric titrations, using methods similar to those described in our previous papers.[6b,c,7] These short double-stranded oligodeoxynucleotides were chosen based on a previous NMR study indicating that berberine has a binding preference for AT-rich double-stranded DNA.[5c] The obtained association constants (K_a's), together with those of Ber-C_{2-4}-Ber with $d(AAGAATTCTT)_2$, $d(TAAGAATTCTTA)_2$, and $d(TTAAGAATTCTTAA)_2$ for comparison, are shown in Table 1.

Several observations can be summarized from the analyses of the association constants. The first finding is that, as observed in the strong binding of Ber-C_{2-4}-Ber to $d(AAGAATTCTT)_2$ and $d(TAAGAATTCTTA)_2$,[7] both Jat-C_{2-4}-Jat and Ber-C_{2-4}-Jat exhibit much higher binding affinities than monomeric berberine or



Scheme 2. Synthetic route for Jat-C_{2-4}-Jat and Ber-C_{2-4}-Jat.

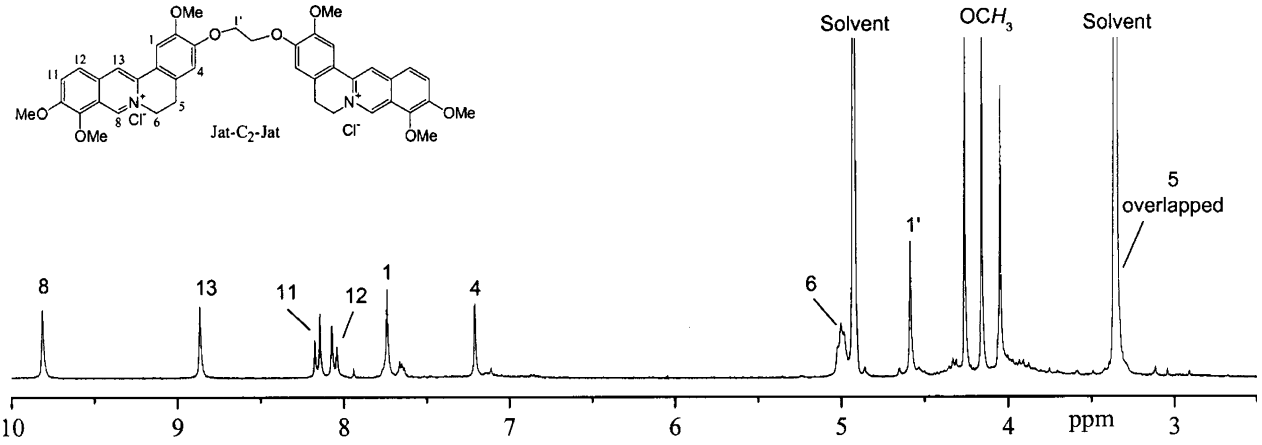


Figure 1. ^{1}H NMR (300 MHz) spectrum of Jat-C_2-Jat in CD_3OD.

Table 1. Association constants (K_a's, M^{-1})[a] of palmatine, berberine, Jat-C_{2-4}-Jat, Ber-C_{2-4}-Jat, and Ber-C_{2-4}-Ber[b] with d(AAGAATTCTT)$_2$, d(TAAGAATTCTTA)$_2$, and d(TTAAGAATTCTTAA)$_2$

Alkaloid	d(AAGAATTCTT)$_2$		d(TAAGAATTCTTA)$_2$		d(TTAAGAATTCTTAA)$_2$	
	K_a	RA[c]	K_a	RA[c]	K_a	RA[c]
Palmatine	$(1.78 \pm 0.06) \times 10^4$	1.0	$(5.34 \pm 0.35) \times 10^4$	1.0	$(4.45 \pm 0.25) \times 10^4$	1.0
Jat-C_2-Jat	$(2.78 \pm 0.41) \times 10^5$	15.6	$(2.94 \pm 0.31) \times 10^5$	5.5	$(4.77 \pm 0.24) \times 10^5$	10.7
Jat-C_3-Jat	$(9.83 \pm 0.70) \times 10^4$	5.5	$(1.60 \pm 0.06) \times 10^5$	3.0	$(2.10 \pm 0.36) \times 10^5$	4.7
Jat-C_4-Jat	$(8.04 \pm 0.73) \times 10^4$	4.5	$(8.63 \pm 0.82) \times 10^4$	1.6	$(2.22 \pm 0.08) \times 10^5$	5.0
Berberine	$(1.24 \pm 0.12) \times 10^4$	1.0	$(2.93 \pm 0.31) \times 10^4$	1.0	$(6.37 \pm 0.22) \times 10^4$	1.0
Ber-C_2-Jat	$(1.38 \pm 0.11) \times 10^5$	11.1	$(4.26 \pm 0.11) \times 10^5$	14.5	$(7.95 \pm 0.70) \times 10^5$	12.5
Ber-C_3-Jat	$(1.32 \pm 0.08) \times 10^5$	10.6	$(4.51 \pm 0.29) \times 10^5$	15.4	$(1.46 \pm 0.29) \times 10^6$	22.9
Ber-C_4-Jat	$(1.05 \pm 0.04) \times 10^5$	8.5	$(2.51 \pm 0.12) \times 10^5$	8.6	$(1.08 \pm 0.08) \times 10^6$	17.0
Ber-C_2-Ber	$(1.18 \pm 0.10) \times 10^5$	9.5	$(1.62 \pm 0.35) \times 10^6$	55.3	$(2.25 \pm 0.16) \times 10^6$	35.3
Ber-C_3-Ber	$(2.46 \pm 0.07) \times 10^5$	19.8	$(2.76 \pm 0.37) \times 10^6$	94.2	$(7.27 \pm 1.13) \times 10^6$	114.1
Ber-C_4-Ber	$(8.78 \pm 0.26) \times 10^4$	7.1	$(3.49 \pm 0.60) \times 10^5$	11.9	$(1.45 \pm 0.08) \times 10^6$	22.8

[a] In 50 mM Tris–HCl (pH 6.35) at room temperature.
[b] The association constants of Ber-C_{2-4}-Ber with d(AAGAATTCTT)$_2$ and d(TAAGAATTCTTA)$_2$ are from Ref. 7.
[c] RA denotes relative affinity. Jat-C_{2-4}-Jat and berberine-containing dimers (i.e., Ber-C_{2-4}-Ber and Ber-C_{2-4}-Jat) are relative to palmatine and berberine, respectively.

palmatine. Jat-C_{2-4}-Jat bind to d(AAGAATTCTT)$_2$ 4- to 16-fold, to d(TAAGAATTCTTA)$_2$ 2- to 6-fold, and to d(TTAAGAATTCTTAA)$_2$ 5- to 11-fold more strongly than palmatine. Ber-C_{2-4}-Jat bind to d(AAGAATTCTT)$_2$ 8- to 11-fold, to d(TAAGAATTCTTA)$_2$ 8- to 15-fold, and to d(TTAAGAATTCTTAA)$_2$ 12- to 23-fold more strongly than berberine. These enhanced DNA-binding abilities may be ascribed to the cooperative interactions of the two protoberberine subunits in these dimers. These results suggest that the dimerization of protoberberine alkaloids is an efficient approach to increase their DNA-binding abilities.[10]

Second, these dimers show a prominent structure–activity correlation with the three oligodeoxynucleotide duplexes. Jat-C_{2-4}-Jat show similar relative binding affinities in the order of Jat-C_2-Jat > Jat-C_3-Jat > Jat-C4-Jat, demonstrating that ethyl chain is the best linker. This is different from Ber-C_{2-4}-Ber in which propyl chain is the most suitable spacer.[7] Whereas Ber-J_{2-4}-Jat have comparable binding abilities, suggesting that they average the effect of jatrorrhizine homodimers and berberine homodimers. These results suggest that the binding affinities of protoberberine dimers can be modulated by varying the spacer lengths.

Third, for the dimers with the same linkers, except the binding of Jat-C_2-Jat to d(AATAATTCTT)$_2$, berberine-containing dimers (i.e., Ber-C_{2-4}-Ber and Ber-C_{2-4}-Jat) show higher binding affinities than jatrorrhizine homodimers (i.e., Jat-C_{2-4}-Jat), mostly in the order of berberine dimers > berberine–jatrorrhizine heterodimers > jatrorrhizine dimers. For example, Ber-C_3-Ber binds to d(TTAAGAATTCTTAA)$_2$ over 5-fold more strongly than Ber-C_3-Jat and ca. 35-fold than Jat-C_3-Jat, respectively. As shown in Table 1, palmatine has comparable DNA-binding affinity with berberine, thus these results indicate that attaching positions have significant impact on the DNA-binding affinities of protoberberine dimers.

The foregoing results may be rationalized by taking into account the structural differences of these dimers. Our recent study has suggested that the quaternary cation in protoberberine plays a key role in the DNA-binding, that is, lack of positively charged center will result in the loss of the binding ability of berberine.[7b] Therefore, the

way how the two charge centers in these protoberberine dimers cooperate with each other will control the binding strength. Thus, the spacer length dependence may be due to the variation of the distances between the two charge centers with spacer lengths. On the other hand, according to the Corey–Pauling–Koltun (CPK) models of these dimers with the same linkers, the distances between the two charge centers are in the order of jatrorrhizine homodimers > berberine–jatrorrhizine heterodimers > berberine homodimers. The greater distances in Jat-C_{2-4}-Jat may make their DNA-binding process more energy-consuming. As a consequence, jatrorrhizine homodimers exhibit weaker binding abilities than berberine-containing dimers. Another factor that should be also taken into account is that the different linking positions may lead to different cooperation of the two charge centers.

The interactions of Jat-C_{2-4}-Jat and Ber-C_{2-4}-Jat with CT DNA were also monitored by absorption spectrometry. Representative spectrophotometric titrations of Jat-C_3-Jat and Ber-C_3-Jat are exemplified in Figure 2. It is observed that the addition of CT DNA to the solutions of the dimers resulted in the large hypochromicities (19.3–35.8%) and bathochromic shifts (1.4–8.6 nm) (Fig. 2 and Table 2). These spectroscopic variations provide unambiguous experimental evidences that Jat-C_{2-4}-Jat and Ber-C_{2-4}-Jat form stable complexes with CT DNA. The extent of spectral changes (especially the hypochromicities), which is related to the strength of binding,[11] indicates that these dimers strongly bind to CT DNA, which is also supported by the association constants obtained from spectrofluorimetric titration.

It should be noted that during the titrations of Ber-C_{2-4}-Jat with CT DNA, well-resolved isosbestic points were observed, revealing the existence of one preferential, almost exclusive, binding mode. Upon addition of CT DNA to Jat-C_{2-4}-Jat, however, no isosbestic points were observed, which may imply that at least two independent binding modes are occupied by Jat-C_{2-4}-Jat when bound to CT DNA.[12]

3. Concluding remarks

Six jatrorrhizine homodimers and berberine–jatrorrhizine heterodimers have been successfully synthesized in moderate to good yields. These protoberberine dimers

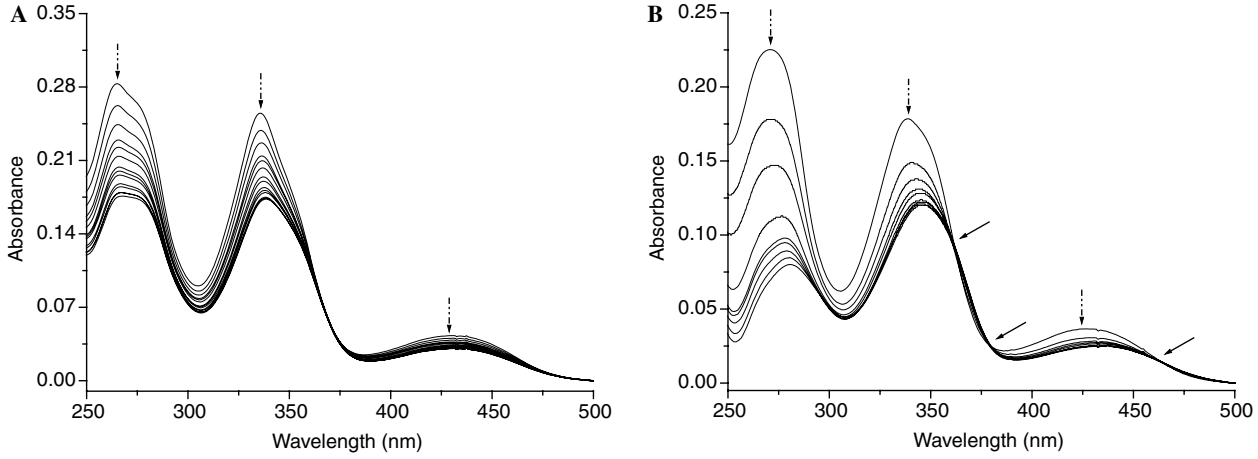


Figure 2. Spectrophotometric titrations of (A) Jat-C_3-Jat (1.11×10^{-5} M) and (B) Ber-C_3-Jat (1.00×10^{-5} M) with CT DNA (0–4.01×10^{-5} M) in 50 mM Tris buffer (pH 6.35) at 25 °C. The dash-dot arrows indicate the decreasing absorption bands during the course of titration; solid arrows indicate the isosbestic points.

Table 2. Association constants (K_a's, M^{-1}) and photophysical properties of berberine, palmatine, Jat-C_{2-4}-Jat, and Ber-C_{2-4}-Jat with CT DNA[a]

Alkaloid	K_a[b]	Hypochromicity[c] (%)	Red shift[c] (nm)	Isosbestic point (nm)
Berberine	$(1.12 \pm 0.04) \times 10^4$	5.0	2.0	354, 382, 440
Palmatine	$(4.17 \pm 0.20) \times 10^3$	9.2	2.5	353, 379, 441[d]
Jat-C_2-Jat	$(7.33 \pm 0.55) \times 10^4$	30.0	6.5	ND[e]
Jat-C_3-Jat	$(9.54 \pm 0.33) \times 10^4$	33.1	2.6	ND[e]
Jat-C_4-Jat	$(5.97 \pm 0.59) \times 10^4$	30.8	1.4	ND[e]
Ber-C_2-Jat	$(1.16 \pm 0.07) \times 10^5$	19.6	5.4	355, 380, 447
Ber-C_3-Jat	$(1.39 \pm 0.08) \times 10^5$	35.8	8.6	363, 378, 461
Ber-C_4-Jat	$(5.87 \pm 0.39) \times 10^4$	19.3	5.0	356, 385, 461

[a] In 50 mM Tris–HCl buffer (pH 6.35) at 25 °C.
[b] Obtained from spectrofluorimetric titration experiment.
[c] Observed at the maxima around 338 nm.
[d] Data from Ref. 5b.
[e] ND means no obvious isosbestic points were detected.

show much higher DNA-binding affinities than their monomeric components, and thus may be exploitable as potentially efficient DNA-binding agents. A comparative study of the DNA-binding behaviors of berberine homodimers, berberine–jatrorrhizine heterodimers, and jatrorrhizine homodimers suggests that there is a possibility of modulating the DNA-binding affinities of protoberberine dimers by adjusting the spacer lengths and linking positions. These findings may provide useful guidance for future rational design of protoberberine derivatives having potentially high DNA-binding affinities. Further efforts aiming at developing strong DNA-binding agents from chemically modified protoberberines are continuing in our laboratories, which will be reported in due course.

4. Experimental

NMR spectra were recorded at Varian Unity INOVA-500 (or 125) or 300 and deuterium solvents were used as an internal reference. HR-ESI-MS spectra were measured on Perkin-Elmer Sciex Api Qstar Pulsar i LCMS. Low resolution ESI MS spectra were measured on Shimadzu LCMS-2010. Fluorescence measurements were made on a Perkin-Elmer Luminescence Spectrometer LS55. Absorption spectra were recorded on a Jasco UV-530 or Shimadzu UV-1601 ultraviolet–visible spectrophotometer.

Oligodeoxynucleotides were purchased from Invitrogen Life Technologies and purified by open reversed-phase ODS column chromatography before use. CT DNA was purchased from Pharmacia (Uppsala, Sweden). The concentrations of single-stranded oligodeoxynucleotides were spectrophotometrically determined, supposing the molar extinction coefficients of A, T, G, and C to be 16,000, 9600, 12,000, and 7000 mol^{-1} dm^3 cm^{-1} at 260 nm, respectively. The molar extinction coefficient used for the concentration determination of CT DNA was approximately 13,200 mol^{-1} dm^3 cm^{-1} in base pair at 260 nm. Single-stranded oligodeoxynucleotides were formed into double-stranded oligodeoxynucleotides, using methods similar to those described in our previous study.[6b] Jatrorrhizine chloride and berberine chloride were extracted from Chinese herbal medicine ('Huang-Lian'). All other synthetic reagents were of analytical grade. Berberrubine (**2**) and Ber-C_{2-4}-Ber were prepared according to the reported methods.[7,9]

4.1. Synthesis of Jat-C_{2-4}-Jat

General procedures: a mixture of **1** (20 mg, 0.054 mmol), K_2CO_3 (70 mg, 0.51 mmol), and 4 Å molecular sieves (20 mg) in anhydrous DMF (1.5 mL) was stirred at 60 °C for 30 min. Then α,ω-dibromoalkane (0.022 mmol) in DMF (0.2 mL) was added dropwise to the reaction mixture. The reaction was carried out at 60 °C under nitrogen atmosphere for 1.5 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated under reduced pressure, dissolved in methanol–water (1/1), and applied to an anion exchange resin (Bio-Rad AG1-X2, chloride form, eluted with 1/1 methanol–water). The fractions containing the product were collected and concentrated under reduced pressure. The resulting residue was purified by chromatography on a basic aluminum oxide column, eluted with $CHCl_3$–CH_3OH (50/1–10/1), to give the desired jatrorrhizine homodimers.

Jat-C_2-Jat: yield 29%, 6 mg. ^{1}H NMR (5/1 $CDCl_3$–CD_3OD, 500 MHz) δ 3.31–3.36 (m, 4H), 4.05 (s, 6H), 4.12 (s, 6H), 4.26 (s, 6H), 4.61 (m, 4H), 4.99 (m, 4H), 7.07 (s, 2H), 7.55 (s, 2H), 7.94–8.01 (m, 4H), 8.69 (s, 2H), 9.79 (s, 2H). ^{13}C NMR (5/1 $CDCl_3$–CD_3OD, 125 MHz) δ 151.2, 150.2, 149.9, 144.9, 144.3, 138.0, 133.5, 127.9, 126.6, 123.1, 121.8, 120.4, 119.3, 112.9, 108.9, 67.8, 62.0, 56.8, 56.6, 56.4, 26.9. HR-ESI-MS for $C_{42}H_{42}N_2O_8$ ($[M-2Cl]^{2+}$): calcd, 351.1471; found, 351.1477.

Jat-C_3-Jat: yield 38%, 8 mg. ^{1}H NMR (5/1 $CDCl_3$–CD_3OD, 500 MHz) δ 2.41 (m, 2H), 3.25–3.28 (t, J = 5.5, 6.0 Hz, 4H), 4.06 (s, 6H), 4.09 (s, 6H), 4.21 (s, 6H), 4.37–4.40 (t, J = 5.5 Hz, 4H), 4.93–4.96 (t, J = 6.0 Hz, 4H), 6.87 (s, 2H), 7.49 (s, 2H), 7.83–7.85 (d, J = 10.0 Hz, 2H), 7.97–7.99 (d, J = 9.0 Hz, 2H), 8.66 (s, 2H), 9.71 (s, 2H). ^{13}C NMR (5/1 $CDCl_3$–CD_3OD, 125 MHz) δ 151.4, 149.9, 149.4, 144.5, 143.8, 137.7, 133.3, 127.7, 126.1, 123.1, 121.4, 119.8, 118.5, 112.2, 108.4, 64.9, 61.7, 56.5, 56.4, 56.0, 29.1, 26.7. HR-ESI-MS for $C_{43}H_{44}N_2O_8$ ($[M-2Cl]^{2+}$): calcd, 358.1549; found, 358.1553.

Jat-C_4-Jat: yield 42%, 9 mg. ^{1}H NMR (5/1 $CDCl_3$–CD_3OD, 500 MHz) δ 2.17 (m, 4H), 3.29 (m, 4H), 4.04 (s, 6H), 4.08 (s, 6H), 4.23 (s, 6H), 4.23 (m, 4H), 4.97 (m, 4H), 6.78 (s, 2H), 7.49 (s, 2H), 7.79–7.80 (d, J = 8.5 Hz, 2H), 7.947–7.96 (d, J = 9.0 Hz, 2H), 8.67 (s, 2H), 9.73 (s, 2H). ^{13}C NMR (5/1 $CDCl_3$–CD_3OD, 125 MHz) δ 151.2, 149.9, 149.4, 144.7, 143.9, 137.9, 133.4, 127.7, 126.0, 123.2, 121.5, 119.8, 118.2, 111.4, 108.3, 68.4, 61.9, 56.6, 56.5, 56.1, 26.8, 24.9. HR-ESI-MS for $C_{44}H_{46}N_2O_8$ ($[M-2Cl]^{2+}$): calcd, 365.1627; found, 365.1610.

4.2. Synthesis of compounds 3–5

General procedures: a solution of **2** (61 mg, 0.17 mmol) and α,ω-dibromoalkane (1.0 mL) in anhydrous DMF (3.0 mL) was stirred at 80 °C for 2 h. After the reaction mixture was cooled to room temperature, diethyl ether (100 mL) was added. The resulting precipitates were collected, dissolved in methanol–water (1/1), and applied to an anion exchange resin (Bio-Rad AG1-X2, chloride form, eluted with 1/1 methanol–water). The fractions containing the product were collected and concentrated under reduced pressure. The crude product was purified by chromatography on a silica gel column, eluted with $CHCl_3$–CH_3OH (10/1), to give **3**–**5** as light yellow solids.

Compound **3**: yield 75%, 59 mg. ESI-MS: m/z 428 ($[M-Cl]^+$). The data of ^{1}H NMR were in full agreement with those published previously.[6c]

Compound **4**: yield 81%, 66 mg. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.42 (m, 2H), 3.21 (t, J = 6.3 Hz, 2H), 3.82 (t, J = 6.6 Hz, 2H), 4.06 (s, 3H), 4.40 (t, J = 6.0 Hz, 2H), 4.94 (t, J = 6.3 Hz, 2H), 6.16 (s, 2H), 7.08 (s, 1H), 7.78 (s, 1H), 8.00 (d, J = 9.3 Hz, 1H), 8.20 (d, J = 9.3 Hz, 1H), 8.93 (s, 1H), 9.78 (s, 1H). ESI-MS: m/z 442 ($[M-Cl]^+$).

Compound **5**: yield 80%, 66 mg. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.98-2.12 (m, 4H), 3.20 (t, J = 6.0 Hz, 2H), 3.67 (t, J = 6.3 Hz, 2H), 4.05 (s, 3H), 4.31 (t, J = 6.3 Hz, 2H), 4.94 (t, J = 6.0 Hz, 2H), 6.16 (s, 2H), 7.07 (s, 1H), 7.78 (s, 1H), 7.99 (d, J = 9.0 Hz, 1H), 8.19 (d, J = 9.0 Hz, 1H), 8.92 (s, 1H), 9.74 (s, 1H). ESI-MS: m/z 456 ($[M-Cl]^+$).

4.3. Synthesis of Ber-C_{2-4}-Jat

General procedures: a mixture of **1** (10 mg, 0.027 mmol), K_2CO_3 (18.5 mg, 0.134 mmol), and 4 Å molecular sieves (10 mg) in anhydrous DMF (0.7 mL) was stirred at 60 °C for 30 min. Then **3** (or **4** or **5**) (0.056 mmol) in anhydrous DMF (2.0 mL) was added dropwise. The reaction was carried out at 60 °C under nitrogen atmosphere for 1.5 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated under reduced pressure, dissolved in methanol–water (1/1), and applied to an anion exchange resin (Bio-Rad AG1-X2, chloride form, eluted with 1/1 methanol–water). The fractions containing the product were collected and concentrated under reduced pressure. The resulting residue was purified by chromatography on a basic aluminum oxide column, eluted with $CHCl_3$–CH_3OH (50/1–10/1), to give the desired berberine–jatrorrhizine heterodimers.

Ber-C_2-Jat: yield 39%, 8 mg. ^{1}H NMR (5/1 $CDCl_3$–CD_3OD, 500 MHz) δ 3.24 (m, 2H), 3.31 (m, 2H) 3.96 (s, 3H), 4.11 (s, 3H), 4.13 (s, 3H), 4.24 (s, 3H), 4.62–4.63 (m, 2H), 4.88–4.95 (m, 6H), 6.08 (s, 2H), 6.85 (s, 1H), 7.11 (s, 1H), 7.48 (s, 1H), 7.64 (s, 1H), 7.95–8.12 (m, 4H), 8.50 (s, 1H), 8.92 (s, 1H), 9.71 (s, 1H), 9.91 (s, 1H). ^{13}C NMR (5/1 $CDCl_3$–CD_3OD, 125 MHz) δ 150.7, 150.6, 150.2, 149.1, 148.4, 144.9, 144.2, 144.0, 142.8, 137.9, 137.8, 133.7, 133.3, 129.9, 128.2, 126.6, 126.3, 123.5, 123.4, 122.3, 121.8, 120.8, 120.0, 119.8, 119.3, 112.5, 109.3, 108.6, 105.0, 102.1, 71.9, 68.1, 61.8, 56.8, 56.3, 56.0, 27.1, 26.6. HR-ESI-MS for $C_{41}H_{38}N_2O_8$ ($[M-2Cl]^{2+}$): calcd, 343.1314; found: 343.1281.

Ber-C_3-Jat: yield 75%, 15 mg. ^{1}H NMR (5/1 $CDCl_3$–CD_3OD_3, 500 MHz) δ 2.54–2.56 (m, 2H), 3.24–3.25 (m, 4H), 4.01 (s, 3H), 4.08 (s, 3H), 4.11 (s, 3H), 4.25 (s, 3H), 4.52–4.54 (t, J = 6 Hz, 2H), 4.66–4.69 (t, J = 6 Hz, 2H), 4.95–5.01 (m, 4H), 6.09 (s, 2H), 6.87 (s, 1H), 7.20 (s, 1H), 7.47 (s, 1H), 7.57 (s, 1H), 7.94–7.99 (m, 3H), 8.05–8.07 (m, 1H), 8.47 (s, 1H), 8.76 (s, 1H), 9.76 (s, 1H), 9.92 (s, 1H). ^{13}C NMR (5/1 $CDCl_3$–CD_3OD_3, 125 MHz) δ 151.5, 150.7, 150.3, 150.1, 149.4, 148.4, 144.8, 144.3, 144.1, 143.5, 138.1, 137.9, 133.6, 133.3, 129.9, 128.2, 126.6, 126.4, 123.1, 122.9, 121.9, 121.7, 120.2, 120.0, 119.8, 118.6, 112.4, 108.6, 108.3, 105.0, 102.0, 71.2, 65.8, 61.8, 56.7, 56.4, 56.2, 55.8, 29.4, 27.1, 26.6. HR-ESI-MS for $C_{42}H_{40}N_2O_8$ ($[M-2Cl]^{2+}$): calcd, 350.1392; found, 350.1361.

Ber-C_4-Jat: yield 46%, 10 mg. ^{1}H NMR (1/1 $CDCl_3$–CD_3OD, 500 MHz) δ 2.21 (m, 2H), 3.27–3.33 (m, 4H), 3.99 (s, 3H), 4.13–4.14 (m, 6H), 4.25 (s, 3H), 4.30 (m, 2H), 4.57 (m, 2H), 4.96–4.97 (m, 4H), 6.12 (s, 2H), 6.91 (s, 1H), 7.60 (s, 1H), 8.00–8.08 (m, 4H), 8.62 (s, 1H), 8.76 (s, 1H), 9.77 (m, 2H). ^{13}C NMR (1/1 $CDCl_3$–CD_3OD, 125 MHz) δ 151.3, 150.5, 150.1, 150.0, 149.2, 148.2, 144.2, 143.9, 143.1, 137.8, 137.8, 133.4, 133.3, 129.6, 127.8, 126.4, 126.3, 122.6, 122.7, 121.7, 121.5, 120.0, 119.8, 119.7, 118.4, 111.7, 108.3, 107.9, 104.8, 101.8, 73.8, 68.5, 61.2, 56.2, 55.7, 55.6, 28.9, 26.7, 26.4, 25.1. HR-ESI-MS for $C_{43}H_{42}N_2O_8$ ($[M-2Cl]^{2+}$): calcd, 357.1471; found: 357.1458.

4.4. Spectrofluorimetric titrations

Spectrofluorimetric titrations were carried out with fixed concentrations of the drugs (i.e., berberine, palmatine, Jat-C_{2-4}-Jat, and Ber-C_{2-4}-Jat), while gradually increasing concentration of the DNA sequences explored in this study. Specifically, to a solution of Jat-C_2-Jat (2.0×10^{-6} M) in 50 mM Tris–HCl buffer (pH 6.35) were added aliquots of d(AAGAATTCTT)$_2$ (1.6×10^{-3} M) solution containing Jat-C_2-Jat (2.0×10^{-6} M) in 50 mM Tris–HCl buffer (pH 6.35). The mixing was achieved by stirring for 5 min. Then the corresponding fluorescence spectra were measured at room temperature using quartz cuvettes of 1.0 cm path (ex 355 nm). The spectrofluorimetric titrations of the other drugs were conducted in a similar way. Association constants (K_a's) were derived from nonlinear curve fitting, using the equation $I = I_0 + ((I_\infty - I_0)/2[B]_0)\{([DNA]_0 + [B]_0 + 1/K_a) - (([DNA]_0 + [B]_0 + 1/K_a)^2 - 4[DNA]_0[B]_0)^{1/2}\}$,[13] wherein $[DNA]_0$ and $[B]_0$ are the initial analytical concentrations of DNA and the drugs, respectively, I, I_0, and I_∞ represent the fluorescence intensities (at 520 nm) of the sample, the drugs alone, and the intensity when the drugs are totally bound, respectively.

4.5. Spectrophotometric titrations

Spectrophotometric titrations were performed with fixed concentrations of the drugs (i.e., Jat-C_{2-4}-Jat and Ber-C_{2-4}-Jat), while gradually increasing concentration of CT DNA. Typically, to a solution of Jat-C_3-Jat (1.11×10^{-5} M) in 50 mM Tris–HCl buffer (pH 6.35) were added aliquots of CT DNA (7.38×10^{-4} M) solution containing Jat-C_3-Jat (1.11×10^{-5} M) in 50 mM Tris–HCl buffer (pH 6.35). The mixing was achieved by stirring for 4 min. Then the corresponding absorption spectra were measured at room temperature using a conventional quartz cell of 1.0 cm path. The spectrophotometric titrations of the other five dimers were conducted in a similar way.

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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.2006.03.004.

References and notes

1. Some reviews on the noncovalent interactions of small molecules with DNA: (a) Dervan, P. B.; Poulin-Kerstien, A. T.; Fechter, E. J.; Edelson, B. S. *Top. Curr. Chem.* **2005**, *253*, 1; (b) Armitage, B. A. *Top. Curr. Chem.* **2005**, *253*, 55; (c) Ihmels, H.; Faulhaber, K.; Viola, G. Evaluation of the DNA-Binding Properties of Cationic Dyes by Absorption and Emission Spectroscopy. In *Highlights in Bioorganic Chemistry: Methods and Applications*; Schmuck, C., Wennemers, H., Eds.; Wiley-VCH: Weinheim, 2004; (d) Gallmeier, H.-C.; Konig, B. *Eur. J. Org. Chem.* **2003**, 3473; (e) Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215.
2. (a) Barton, J. K. *Science* **1986**, *233*, 727; (b) Chrisey, L. A.; Bonjar, G. H. S.; Hecht, S. M. *J. Am. Chem. Soc.* **1988**, *110*, 644; (c) Zein, N.; Sinha, A. M.; McGarhren, W. J.; Ellestad, G. A. *Science* **1988**, *240*, 1198; (d) Wolkenberg, S. E.; Bogor, D. L. *Chem. Rev.* **2002**, *102*, 2477.
3. (a) Creasey, W. A. *Biochem. Pharmacol.* **1979**, *28*, 1081; (b) Birdsall, T. C.; Kelley, G. S. *Altern. Med. Rev.* **1997**, *2*, 94; (c) Lau, C. W.; Yao, X. Q.; Chen, Z. Y.; Kou, W. H.; Huang, Y. *Cardiovasc. Drug Rev.* **2001**, *19*, 234; (d) Vollekova, A.; Kostalova, D.; Kettmann, V.; Toth, J. *Phytother. Res.* **2003**, *17*, 834.
4. (a) Kuo, C. L.; Chou, C. C.; Yung, B. Y. M. *Cancer Lett.* **1995**, *93*, 193; (b) Mutoh, M.; Koshiji, M.; Akao, S.; Fujiwara, H. *J. Ethnopharmacol.* **1999**, *66*, 227; (c) Lin, J. G.; Chung, J. G.; Wu, L. T.; Chen, G. W.; Chang, H. L.; Wang, T. F. *Am. J. Chin. Med.* **1999**, *27*, 265; (d) Krishnan, P. K.; Bastow, K. F. *Anti-Cancer Drug Des.* **2000**, *15*, 255; (e) Fukuda, K.; Hibiya, Y.; Iizuka, N.; Miyamoto, K.; Hazama, S.; Yoshino, S.; Yoshimura, K.; Okita, K.; Fukumoto, T.; Yamamoto, S.; Tangoku, A.; Oka, M. *Cancer Lett.* **2000**, *158*, 35.
5. (a) Krey, A. K.; Halm, F. E. *Science* **1969**, *166*, 755; (b) Kluza, J.; Baldeyron, B.; Colson, P.; Rasoanaivo, P.; Mambu, L.; Frappier, F.; Bailly, C. *Eur. J. Pharm. Sci.* **2003**, *20*, 383; (c) Mazzini, S.; Bellucci, M. C.; Mondelli, R. *Bioorg. Med. Chem.* **2003**, *11*, 505; (d) Yadav, R. C.; Kumar, G. S.; Bhadra, K.; Giri, P.; Sinha, R.; Pal, S.; Maiti, M. *Bioorg. Med. Chem.* **2005**, *13*, 165.
6. (a) Chen, W.-H.; Chan, C.-L.; Cai, Z.; Luo, G.-A.; Jiang, Z.-H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4955; (b) Chen, W.-H.; Qin, Y.; Cai, Z.-W.; Chan, C.-L.; Luo, G.-A.; Jiang, Z.-H. *Bioorg. Med. Chem.* **2005**, *13*, 1859; (c) Pang, J.-Y.; Qin, Y.; Chen, W.-H.; Luo, G.-A.; Jiang, Z.-H. *Bioorg. Med. Chem.* **2005**, *13*, 5835.
7. (a) Chen, W.-H.; Pang, J.-Y.; Qin, Y.; Peng, Q.; Cai, Z.; Jiang, Z.-H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2689; (b) Qin, Y.; Pang, J.-Y.; Chen, W.-H.; Cai, Z.; Jiang, Z.-H. *Bioorg. Med. Chem.* **2006**, *14*, 25.
8. Hirakawa, K.; Kawanishi, S.; Hirano, T. *Chem. Res. Toxicol.* **2005**, *18*, 1545.
9. Iwasa, K.; Kamigauchi, M.; Ueki, M.; Taniguchi, M. *Eur. J. Med. Chem.* **1996**, *31*, 469.
10. (a) Gaugain, B.; Barbet, J.; Capelle, N.; Roques, B. P.; Le Pecq, J. B.; Le Bert, M. *Biochemistry* **1978**, *17*, 5078; (b) Le Pecq, J. B.; Le Bert, M.; Barbet, J.; Roques, B. P. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2915.
11. (a) Long, E. C.; Barton, J. K. *Acc. Chem. Res.* **1990**, *23*, 271; (b) Pyle, C. V.; Rehmann, J. P.; Meshoyrer, R.; Kumer, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051.
12. Palmatine, berberine, and berberine homodimers are reported to interact with double-stranded DNA in an intercalating mode. See Refs. 5b and 7b.
13. Schneider, H.-J.; Yatsimirski, A. K. In *Principles and Methods in Supramolecular Chemistry*; J. Wiley: New York, 2000; pp 137–143.