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Original article

Synthesis and biological evaluation of dialkylaminoalkylamino benzo[*c*][1,7] and [1,8]phenanthrolines as antiproliferative agents

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ABSTRACT

Benzo[*c*][1,7] and [1,8]phenanthroline substituted by dialkylaminoalkyl side chains at position C2 and C1, respectively, were synthesized and their biological activity evaluated. These compounds displayed more potent cytotoxicity toward L1210 cells than the parent unsubstituted compounds, associated with strong DNA interaction. The moderate TopoI inhibitory activity induced by the novel compounds suggests that other cellular targets should be responsible for the antiproliferative activity.

1. Introduction

DNA topoisomerases constitute a superfamily of enzymes which interfere with DNA [1]. These enzymes are essential in every living cell and represent the Nature's solution to the topological problems associated with manipulating double-stranded helical DNA during essential cellular processes, such as replication, transcription, recombination, as well as chromosome compaction and segregation [2]. The main classification of these enzymes is based on their reaction mechanism, *i.e.* their ability to create a single-stranded (type I) or double-stranded (type II) nick in DNA [3]. Among these enzymes, the human DNA topoisomerase IB (TopoI) is of particular interest. It catalyses the major DNA topoisomerase I activity and represents the cellular target for the camptothecin-derived anticancer drugs irinotecan and topotecan currently used in the clinic, but also to many other cellular poisons [4,5]. However, the camptothecin derivatives suffer from several drawbacks, including poor water solubility, lactone instability, prolonged infusion times, and facile apparition of multidrug resistance due to transporter-mediated cellular efflux in cancer cells [6]. As a result, there is a great interest in the development of non-camptothecin topoisomerase I inhibitors as anticancer agents [7].

We previously reported the preparation of non-charged bio-isosters of the benzo[*c*]phenanthridine alkaloids nitidine **1** and fagaronine **2**, in the benzo[*c*][1,7]- and [1,8] phenanthroline (1,7-BZP and 1,8-BZP) series (Scheme 1). Biological evaluations of these compounds showed interesting cytotoxic activities associated with a moderate TopoI inhibition [8]. In a continuation of our work, we report here the synthesis and biological activities of new series of 1,7-BZP and 1,8-BZP bearing at positions C2 and C1 on cycle A, respectively, different dialkylaminoalkyl substituents. These side chains, which mimick natural DNA ligands such as spermine and spermidine, were chosen due to the excellent results reported with such substituents in several related series including indénoisoquinoléines [9], dibenzo[*c*,*h*] [1,6]naphthyridinones [10], camptothécine [11], and acronycine analogs [12].

2. Chemistry

The synthetic route followed to prepare the different compounds was partially reported previously. Briefly, the strategy involved six steps with a benzyne mediated cyclization as the key step. To clarify the lecture, the synthetic plan is divided in two parts: one for the 1,7-BZP series (Scheme 2) and one for the 1,8-BZP

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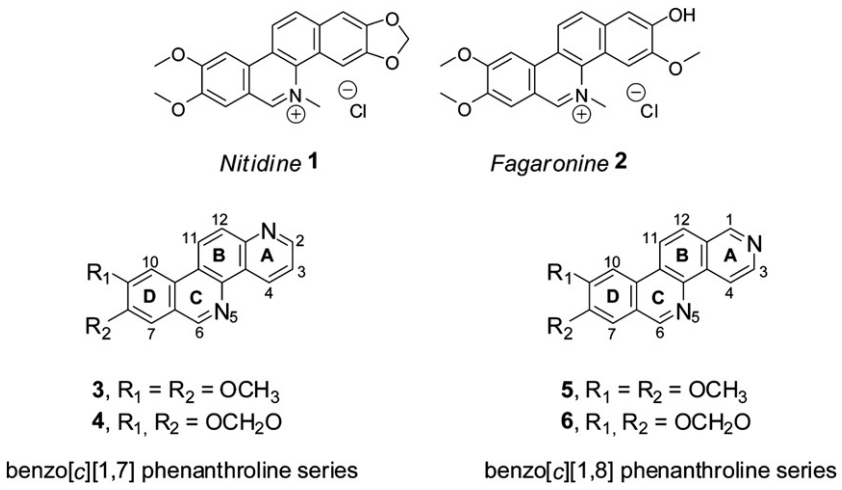


Scheme 1.

series (Scheme 3). Condensation of the 2-bromo- veratraldehyde **7** or piperonal **8** with the 5-amino-quinoline **9** (Scheme 2) or isoquinoline **10** (Scheme 3) gave access to the corresponding Schiff bases. After reduction of the imine function with $NaBH_4$, treatment with an excess of LDA permitted to ensure the cyclization of cycle C [13]. A spontaneous air oxidation step achieved the preparation of the two different phenanthroline cores [14]. Selective introduction of aminoalkyl side chains in α position to the nitrogen atom on cycle A was realized in three steps. Oxidation with *m*-CPBA afforded the BZP *N*-oxide, which were further converted into the chloro derivatives by reaction with $POCl_3$ [15]. Finally, direct heating of the chloro BZP in the desired dialkylaminoalkylamine afforded, after careful column chromatography, the corresponding substituted BZP in low to moderate yield (6–52%).

3. Biological evaluation

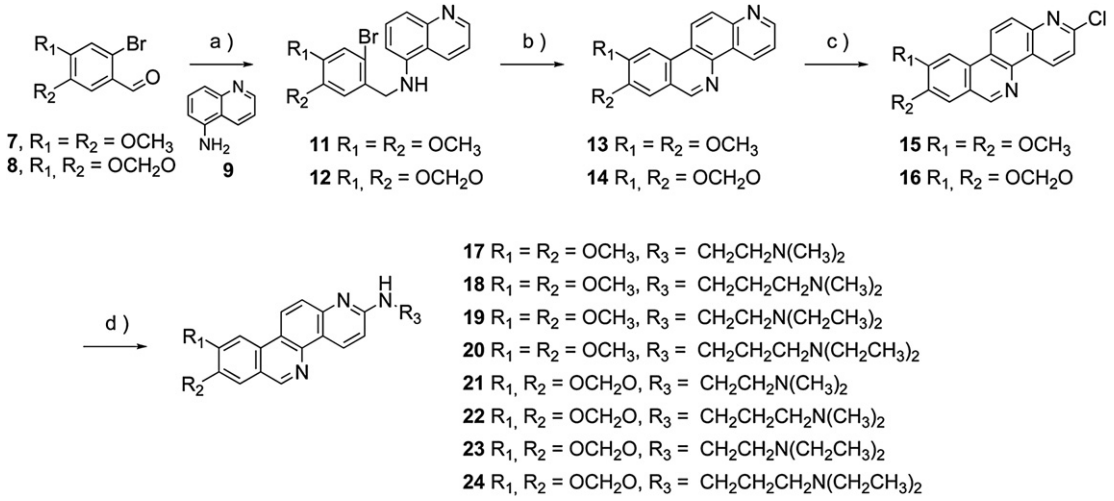
3.1. Evaluation of the cytotoxicity

The study of the cytotoxic properties of new compounds (**17**–**24** and **31**–**36**) was carried out *in vitro* on the L1210 murine leukemic cell line [16]. The results (IC_{50}) are reported in Table 1. Both series exhibited cytotoxicities within the micromolar range. Moreover all compounds presented an increased activity when compared to the parent compounds **3**–**6**. As previously observed in analogous series, methylenedioxy substituted compounds displayed more potent antiproliferative activity than their dimethoxy counterparts. It should also be noted that no straightforward correlation was observed between the side chain length and the cytotoxicity.

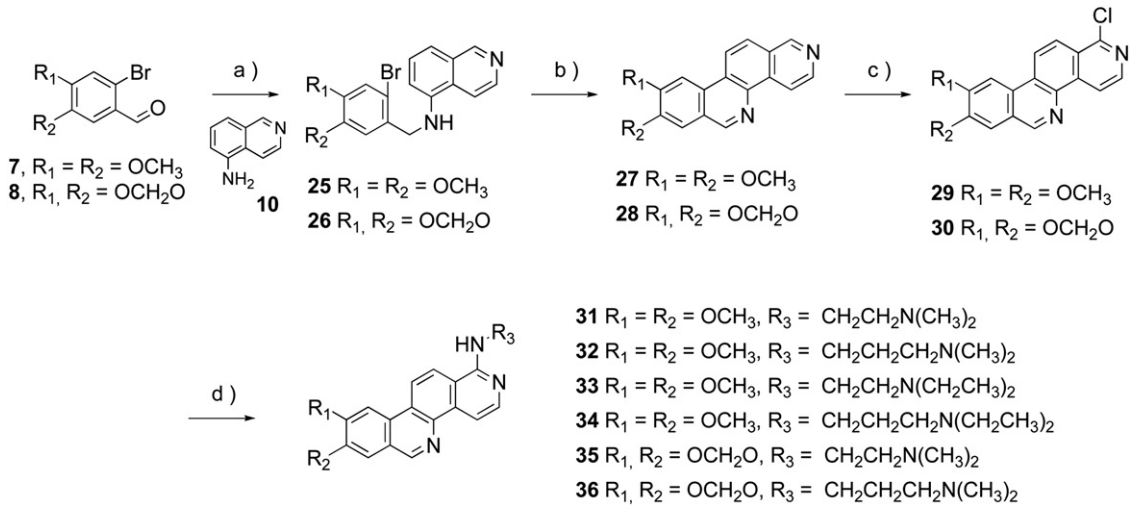
3.2. DNA binding properties and inhibition of topoisomerases I and II

3.2.1. UV–visible and fluorescence spectra

In order to address the DNA binding properties of the novel 1,7-BZP and 1,8-BZP derivatives, the UV–visible absorbance (Fig. 1) and fluorescence spectra (Fig. 2) of the various compounds in the absence and presence of calf thymus DNA were studied [17]. The binding of the drugs to DNA resulted in a decrease of absorbance in the DNA-compound complex spectra (hypochromic shift) at maximum wavelengths depending on the series, 265 nm for 1,8-BZP, 275 nm for dimethoxy-1,7-BZP, and 285 nm for methylenedioxy-1,7-BZP, associated with a small bathochromic shift (see horizontal arrows, shifts are around 10 nm). Concerning the fluorescence



Scheme 2. Benzo[*c*][1,7]phenanthroline series. Reagents and conditions: (a) (i) toluene, reflux; (ii) $NaBH_4$, MeOH, 0 °C; (b) (i) LDA, THF, −78 °C to r.t.; (ii) air oxidation; (c) (i) *m*CPBA, CH_2Cl_2, 0 °C; (iii) $POCl_3$, δ; (d) $H_2N(CH)_nN(R_3)_2$.



Scheme 3. Benzo[*c*][1,8]phenanthroline series. Reagents and conditions: (a) (i) toluene, reflux; (ii) $NaBH_4$, MeOH, 0 °C; (b) (i) LDA, THF, −78 °C to r.t.; (ii) air oxidation; (c) (i) *m*CPBA, CH_2Cl_2, 0 °C; (iii) $POCl_3$, δ; (d) $H_2N(CH)_nN(R_3)_2$.

spectral measurement, DNA binding activities of BZP series were evidenced by a blue shift. In 1,7-BZP series this effect is accompanied by a decrease of the fluorescence intensity. In 1,8-BZP series the observed blue shift is associated with an increase or a decrease of the peak intensity for compounds bearing a methylenedioxy substituent or a dimethoxy substituent, respectively.

3.2.2. DNA melting temperature

The relative DNA affinity of these compounds was assessed using CT-DNA melting temperature studies (Table 2). The ability of the drugs to protect calf thymus DNA (CT*a* DNA, 42% GC bp) against thermal denaturation was used as an indication of their capacity to bind to DNA and to stabilize the DNA double helix. The induced variation of melting temperature evidenced some strong DNA stabilizing agents (**17**, **24**, **36**) with an increase of more than 10 °C of CT-DNA melting temperature. Others compounds are medium or low binders with ΔT_m being 4 to 9 °C. TM value could not be determined for some compounds (ND) due to their poor stability at high temperature, leading to a modification of their absorbance value at DNA wavelength (260 nm). Without focusing on those latter compounds, BZP analogs inducing the highest stabilization of the DNA helix are also those that induced the highest changes in UV/visible absorbance spectra in the absence or presence of CT-DNA (Fig. 1, see compounds **17**, **24** and **36**). Particularly, in dimethoxy-1,7-BZP, compound **17**, bearing a dimethylaminoethylamine chain branched at position 2 efficiently stabilizes the DNA helix, whereas substitution with a longer dimethylaminopropylamine chain results in a loss of DNA stabilization. With compounds bearing a methylenedioxy substituent, a better DNA binding propensity is obtained using a diethylaminopropylamine side chain. In the 1,8-BZP series, DNA stabilization also depends on the nature of the side chain. A better relative affinity is obtained when methylenedioxy-bearing compounds are substituted with a dimethyl aminopropylamine side chain. It appears from these data that both series of BZP display significant DNA binding properties.

Table 1
Cytotoxicities against L1210 cell line.

Compound	cytotoxicity (IC_{50} in μM)	Compound	Cytotoxicity (IC_{50} in μM)
17	1.73	**31**	3.51
18	2.85	**32**	1.80
19	0.96	**33**	1.77
20	1.26	**34**	3.70
21	0.32	**35**	ND
22	0.51	**36**	0.29
23	1.74	**29**	4.30
24	0.83	Fagaronine (2)	6.60
3	7.10	**5**	6.60
4	8.80	**6**	4.40

3.2.3. Circular dichroism measurement

The binding mode of the various BZP analogs was then studied using circular dichroism (Fig. 3). CD spectra of the CT-DNA obtained in the presence of a given concentration of the tested compound (pointed lanes) were compared to the spectra of the CT-DNA alone (dashed lanes) and that of the compound alone (plain lanes). As evidenced from Fig. 3, most of the compounds increased the peak intensities of CT-DNA (negative peak at 245 nm and positive peak at 280 nm), suggesting that they change the super-helicity of DNA. With some compounds, a strong negative induced CD (ICD) below 300 nm was observed, suggesting DNA intercalation, whereas an interesting positive ICD was observed at *ca.* 350 nm with several methylenedioxy-1,7-BZP derivatives, including **22**, **24**, **28** and **36**. Such positive ICD should be attributed to some minor groove binding of the amino side chain in the methylenedioxy-1,7-BZP series.

3.2.4. Topoisomerases I and II inhibition assays

In numerous cases efficient DNA binders, structurally related to 1,7-BZP and 1,8-BZP, have been shown to inhibit DNA dependant enzymes, such as topoisomerases I and/or II [11]. The DNA relaxation induced by topoisomerase I was first studied (Fig. 4). Incubation of increasing concentrations of the various drugs with supercoiled plasmid DNA in the presence of topoisomerase I permitted to characterize the strongest DNA intercalators (**17**, **21**, **22**, **23**, **35** and **36**), evidenced by formation of relaxed plasmid DNA. Compounds bearing a methylenedioxy substituent appeared to be more potent DNA intercalators than their dimethoxy counterparts suggesting that the additional oxirane ring present in the methylenedioxy compounds should increase their DNA intercalation potency. Also, it should be noted that at the lower concentrations (1–5 μM), which are within the cytotoxic values of the various drugs, no significant inhibition of topoismerase I was observed.

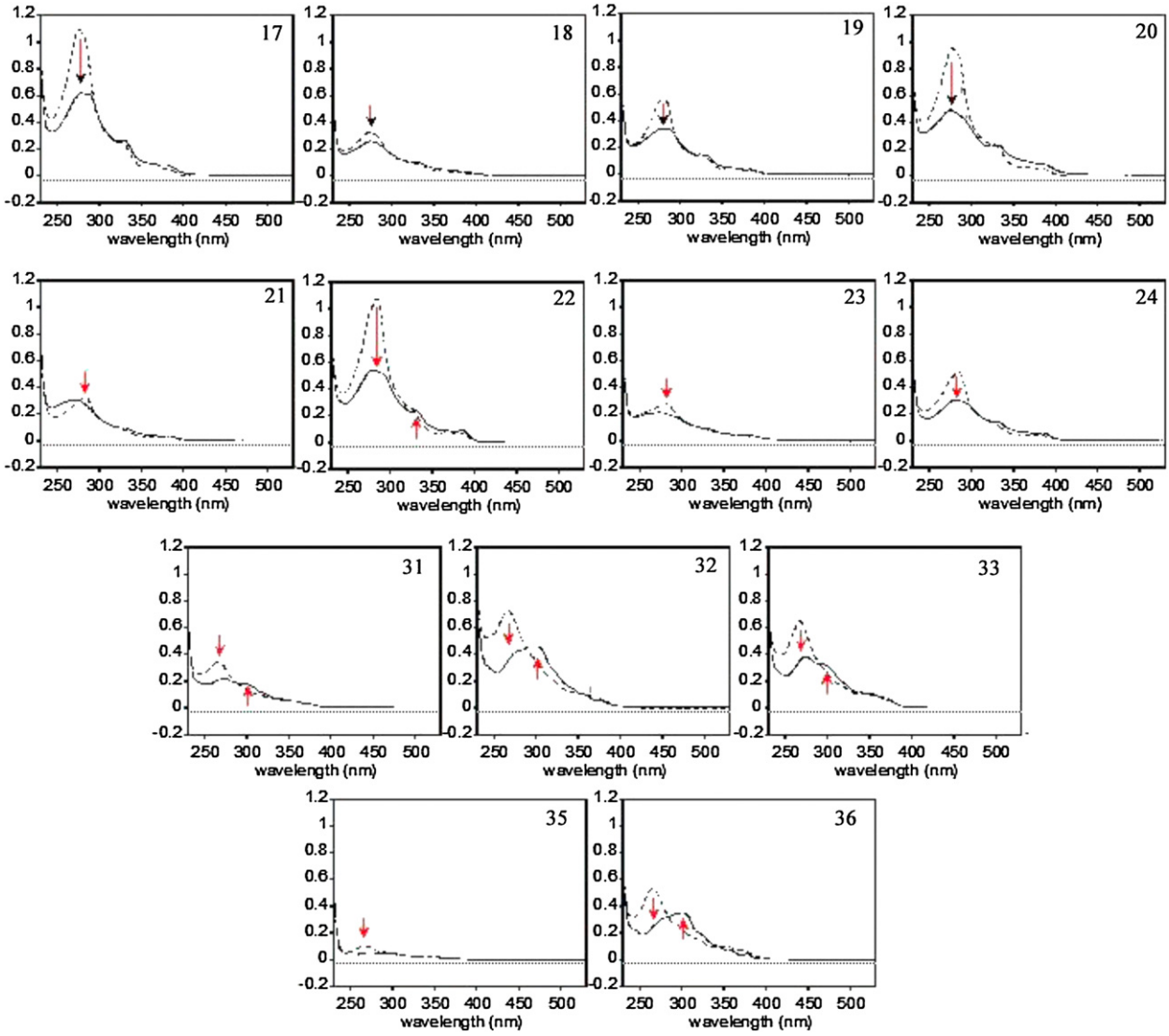


Fig. 1. UV spectra for compounds **17**–**24**, **31**–**33**, and **35**–**36** (20 μM) in the absence (dashed lanes) or presence of CT-DNA (plain lanes) (20 μM). Spectra were recorded in BPE buffer, pH 7.1.

A conventional DNA cleavage assay was used to assess the effects of the new compounds on the catalytic activity of human topoisomerases I and II (Fig. 5). In these experiments, supercoiled plasmid DNA was treated with either topoisomerase I or II in the presence of two concentrations of test drug, CPT and etoposide, respectively, and the DNA relaxation products were then resolved by gel electrophoresis on agarose gel [18]. Fig. 5a evidences that, in contrast with CPT used as a positive control, none of those compounds present TopoI poisoning effects based both on the lack of generated nicked forms from supercoiled circular DNA and the generation of cleaved DNA from a radio-labeled DNA fragment (Fig. 6). In the same way, a weak TopoII poisoning effect was obtained in comparison with the reference compound etoposide (Fig. 5b). As previously noted with the unsubstituted parent compounds, topoisomerases inhibition does not constitute a primary event responsible for the cytotoxic activity.

3.2.5. Sequence-specific DNA binding

Compounds **22** and **24** (and, to a lesser extend, **21** and **23**) gave positive induced circular dichroism in the presence of DNA, that could evidenced for DNA groove binding (Fig. 3). The DNA relaxation profiles of those compounds also suggested some intercalation. Consequently, compounds **22** and **24** were selected to explore their potency to bind DNA in a sequence-specific manner using DNase I footprinting analysis (Fig. 7). The nature of the sequence preferentially recognized by these compounds was determined using a radiolabeled 265-bp DNA restriction fragment as substrate. Comparison of the DNase I digested DNA lanes (lanes "0") with those obtained from DNA previously incubated with increasing concentrations of either of the two compounds evidenced a global decrease in the intensities of all bands. Interestingly, fine quantification of the bands by densitometric analysis suggested some sequence preferences (grey bars) using both **22** and **24**. The protected sequences contained in both cases a CT dinucleotide with any base pairs surrounding it to cover a larger sequence. This result suggests that both **22** and **24** intercalate preferentially at 5′-CT dinucleotide with their side chains lying in the DNA groove, thus resulting in a widening of the covered sequence.

4. Conclusion

In order to improve the antiproliferative profile of 1,7- and 1,8-BZP, dialkylaminoalkyl side chains were introduced on cycle A at position C2 or C1, depending on the series. This substitution of the basic BZP core resulted in a significant improvement of the cytotoxicity. However, the TopoI inhibitory activity induced by the

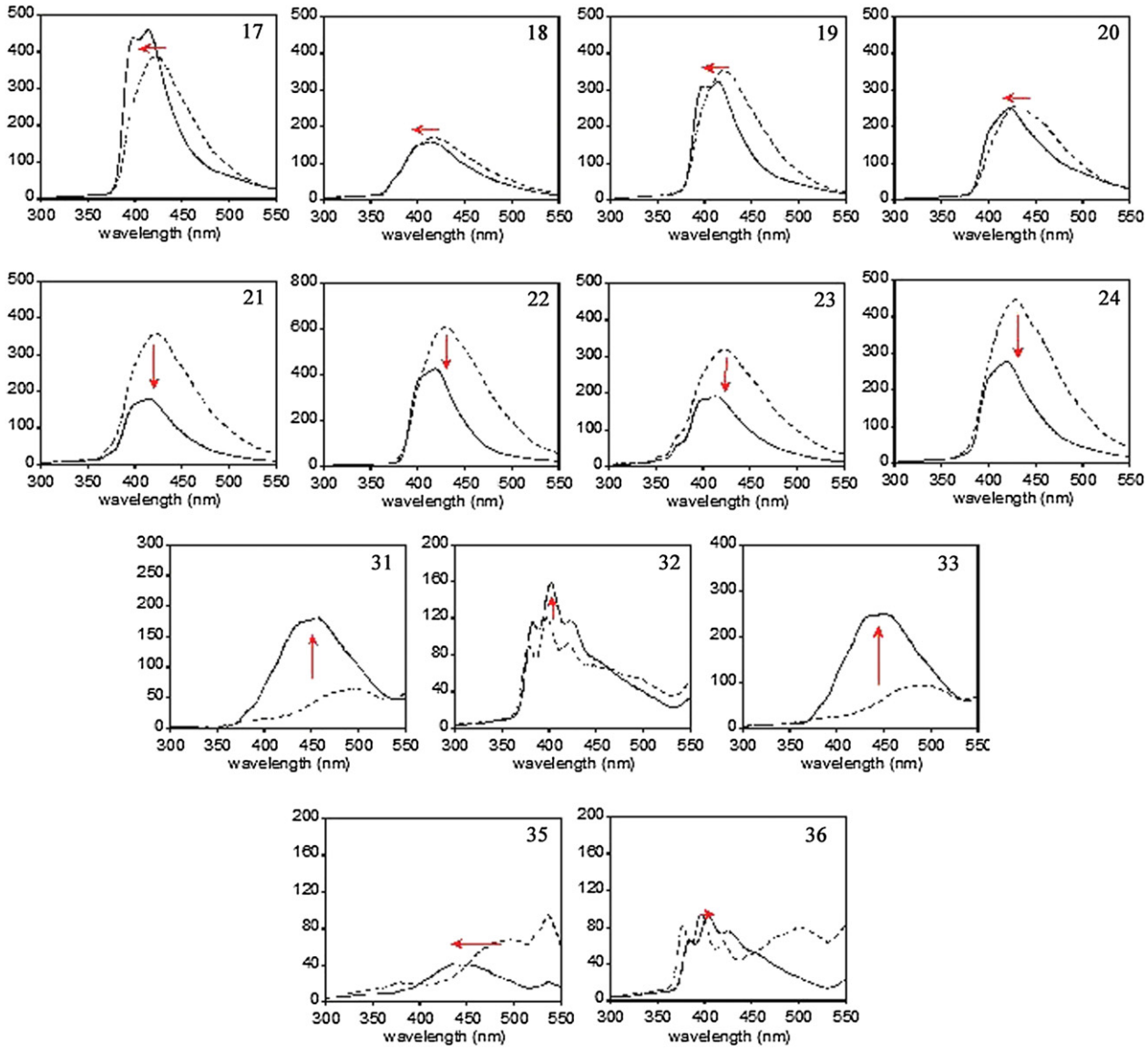


Fig. 2. Fluorescence spectra for compounds **17**–**24**, **31**–**33**, and **35**–**36.** Fluorescence spectral measurement were recorded using 20 μM of the fluorescent in the presence (plain lanes) or absence (dashed lanes) of 200 μM of CT-DNA The excitation wavelengths were 268 nm for compound **35**, 275 nm for compounds **31**, **32**, **33** and **36**, and 285 nm for all other evaluated compounds. The emission spectra were scanned at the wavelength specified in the abscissa of the figures.

novel compounds remains low, suggesting that other cellular targets should be responsible for the antiproliferative activity. It is interesting to underline that similar discrepancies between cytotoxicity and TopoI inhibition have been previously observed in several related series, including camptothecin, anthracenes, aromathecins and indénoisoquinoléines [19]. It is also worth noting that in the indenoisoquinoline and dibenzo[*c*,*h*][1,6]naphthyridine series, the aminoalkyl residue were introduced in cycle B or C [7].

From a structure-activity viewpoint, we had previously shown that the dioxygenated aromatic ring A of natural benzo[*c*]phenanthridines, such as nitidine and fagaronine, could be replaced by a pyridin unit without loss of activity. Introduction of a dialkylaminoalkyl chain in the pyridin cycle A results in an increase of the antiproliferative activity of about one order of magnitude when compared to the parent compounds.

Table 2
CT-DNA melting temperature study.

Compound	ΔT_m CT-DNA (°C)[a]	Compound	ΔT_m CT-DNA (°C)[a]
17	12	**31**	9
18	0	**32**	ND
19	5	**33**	9
20	ND	**34**	ND
21	4	**35**	7
22	ND	**36**	14
23	5	**37**	ND
24	11		

[a] Variation in melting temperature ΔT_m ($T_m^{\text{drug–DNA complex}} - T_m^{\text{DNA alone}}$). Drug/CT DNA ratio = 1.

5. Experimental section

5.1. Chemistry

5.1.1. General

All reactions were carried out in a dried glassware under an argon atmosphere. All solvents were purchased with an analytical grade from SDS. CH_2Cl_2 was distilled from CaH_2. Methanol, acetonitrile and DMF were dried over molecular sieves. All other commercially

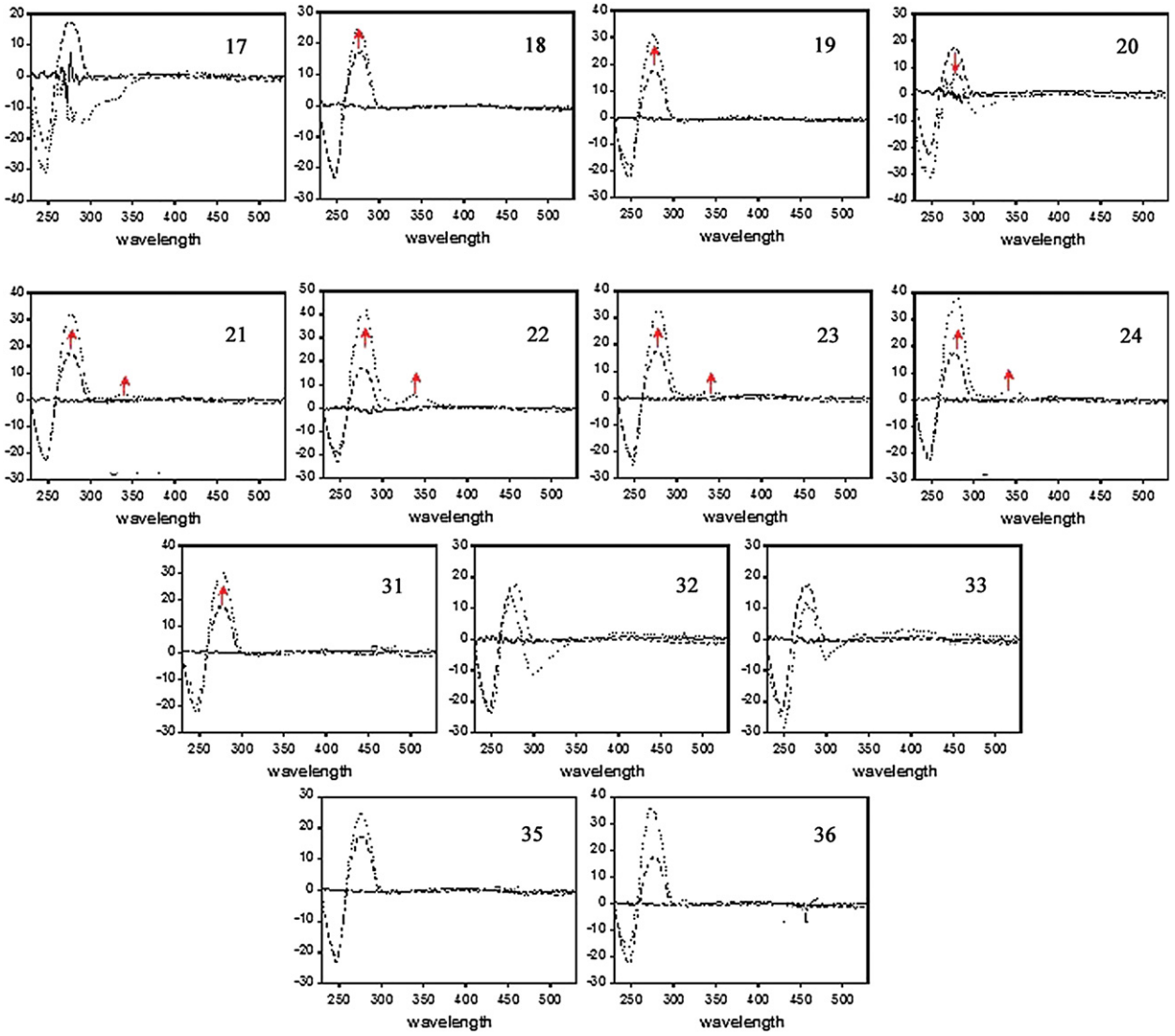


Fig. 3. Circular dichroism spectra for compounds **17**–**24**, **31**–**33**, and **35**–**36** (50 μM) alone (plaine lanes), with CT-DNA (200 μM) (pointed lanes) or CT-DNA (200 μM) alone (dashed lanes). The spectra were recorded from 500 to 230 nm with a resolution of 0.1 nm.

available reagents were used as received. Flash chromatography was performed with silica gel CHROMATOGEL 60 (particle size 20–45 μm or 35–70 μm) supplied by SDS. Yield refers to chromatography and spectroscopically pure compounds, unless otherwise noted. ^{1}H NMR spectra were recorded on a BRUCKER AC 300 MHz spectrometer or a BRUCKER AVANCE 400 MHz spectrometer. Chemical shifts are reported in ppm and corrected to δ_H 7.26 for $CDCl_3$ as internal reference, coupling constants (J) in Hertz. ^{13}C NMR spectra were recorded on a BRUCKER AC 300 spectrometer at 75 MHz. Chemical shifts are reported in ppm and corrected to δ_C 77.16 for $CDCl_3$ as internal reference. The assignments are based upon 1D and 2D spectra recorded using the following pulse sequences from the Bruker standard pulse program library: DEPT, COSY, HSQC, HMBC and NOESY. Mass spectra were measured with a ZQ 2000 Waters mass spectrometer (ESI). High-resolution mass spectra were obtained on a Q-ToF1 ESI mass spectrometer (Waters). Infrared spectra were recorded on a Nicolet FT-IR spectrometer and wavelengths (υ) are given in cm^{-1}.

*5.1.2. General procedure for the preparation of benzo[c] phenanthrolines cores **12**, **13**, **27** and **28***

See Ref. [8].

5.1.3. General procedure for the preparation of benzo[c] phenanthrolines-N-oxides

To a solution of the benzo[*c*]phenanthroline (191 mg, 0.656 mmol) in anhydrous CH_2Cl_2 (10 ml) was added mCPBA (113 mg, 0.656 mmol) at rt. After 3 h the precipitate formed was filtered, washed with a minimum of CH_2Cl_2 then with water and dried. The corresponding N-oxide was used without further purification.

5.1.3.1. 8,9-Dimethoxybenzo[c][1,7]phenanthroline-N-oxide. Yield 79%. IR 2960 (ν CH), 2920, 1642, 1519 (ν CNO), 1478, 1427, 1276, 1178, 1168, 873, 771 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1H), 9.27 (d, 1H, $J = 8.0$ Hz), 8.86 (d, 1H, $J = 8.0$ Hz), 8.76 (d, 1H, $J = 9.0$ Hz), 8.68 (d, 1H, $J = 6.0$ Hz), 7.95 (s, 1H), 7.58 (dd, 1H, $J = 6.0$, 8.0 Hz), 7.46 (s, 1H), 4.20 (s, 3H), 4.09 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6, 151.4, 150.9, 140.9, 140.0, 136.5, 130.3, 128.2, 125.0, 123.8, 123.2, 121.9, 121.6, 117.5, 107.2, 101.7. 56.3 (2 C). MS (ES^+) m/z 329 $[M + Na]^+$. HRMS (ES^+) m/z calcd for $C_{18}H_{15}N_2O_3$ $[M + H]^+$ 307.1083, found 307.1081.

5.1.3.2. 8,9-Methylenedioxybenzo[c][1,7]phenanthroline-N-oxidepar-a>Yield 74%. IR 3093, 2920 (ν CH), 1598, 1473 (ν CNO), 1444, 1265 (ν CN), 1211, 1158, 1032, 946, 821, 780 cm^{-1}. ^{1}H NMR (400 MHz,

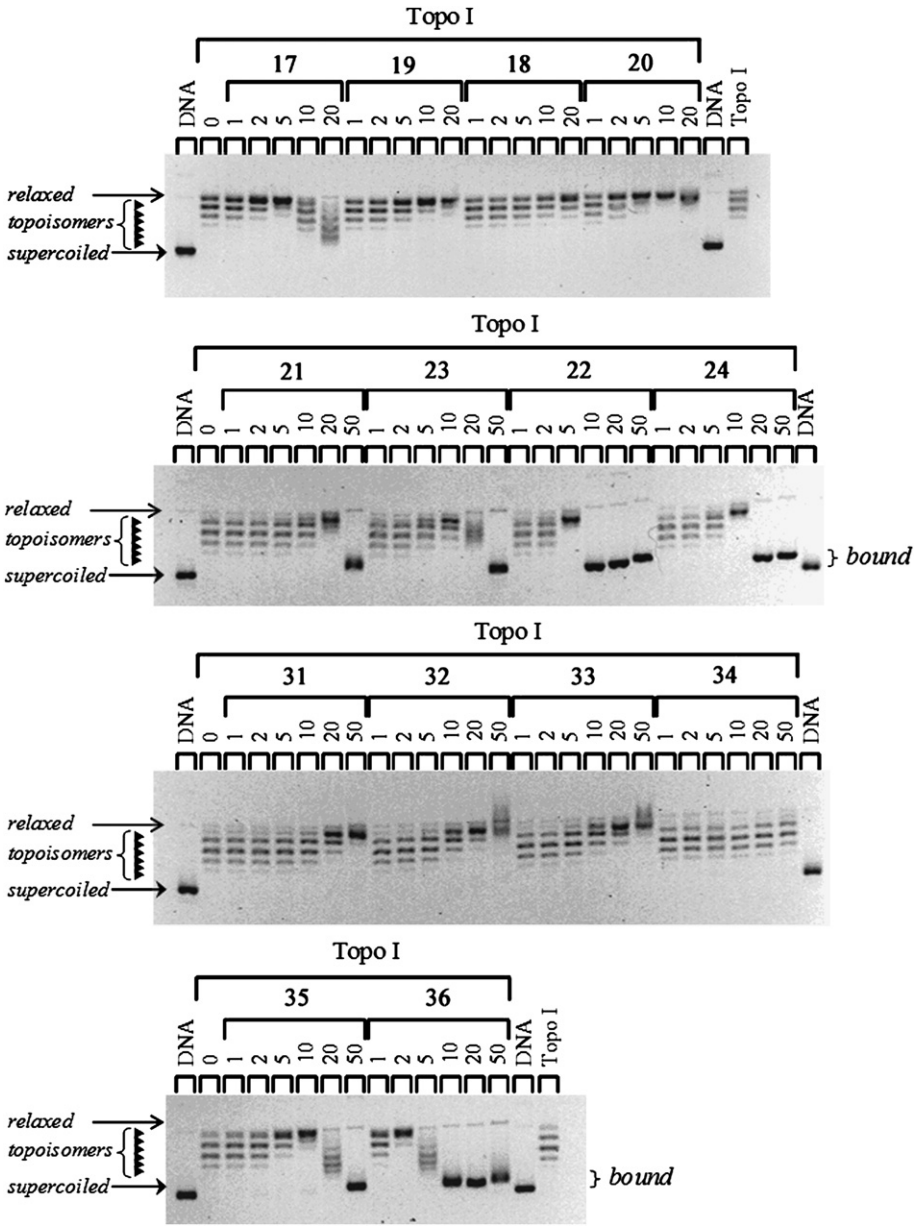


Fig. 4. Topoisomerase I-induced DNA relaxation. Effect of compounds **17**–**24** and **31**–**36** on the relaxation of plasmid DNA by human topoisomerase I. Native supercoiled pUC19 (130 ng, lane DNA) was incubated with 4 units of topoisomerase I in the absence (lane 0) or presence of tested compound at the indicated concentration (1–50 μM). Samples were separated by electrophoresis on a 1% agarose gel, which was stained with ethidium bromide after DNA migration. Gels were photographed under UV light: Nck, nicked; Sc, supercoiled; Rel, relaxed; Topo, topoisomer products.

$CDCl_3$) δ 9.30 (s, 1H), 9.26 (d, 1H, J = 8.5 Hz), 8.90 (d, 1H, J = 9.3 Hz), 8.70 (dd, 1H, J = 6.0, 1.0 Hz), 8.68 (d, 1H, J = 9.3 Hz), 8.01 (s, 1H), 7.56 (dd, 1H, J = 8.5, 6.0 Hz), 7.47 (s, 1H), 6.24 (s, 2H). MS (ES+) m/z 291 $[M+H]^+$, 313 $[M+Na]^+$. HRMS (ES^+) m/z calcd for $C_{17}H_{11}N_2O_3$ $[M+H]^+$ 291.0770, found 291.0766.

5.1.3.3. 8,9-Dimethoxybenzo[c][1,8]phenanthroline-N-oxide. Yield 86%. IR 3070, 2948 (ν CH), 2920, 1733, 1610 (ν CNO), 1519, 1406, 1273 (ν CN), 1227, 1157, 1090, 799 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 9.34 (s, 1H), 9.19 (d, 1H, J = 7.0 Hz), 9.07 (d, 1H, J = 1.5 Hz), 8.61 (d, 1H, J = 9.0 Hz), 8.49 (dd, 1H, J = 7.0, 1.5 Hz), 7.91 (s, 1H), 7.89 (d, 1H, J = 9.0 Hz), 7.47 (s, 1H), 4.21 (s, 3H), 4.14 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.1, 151.5, 151.0, 138.2, 137.3, 136.0, 133.3, 130.5, 128.9, 124.2, 122.8, 122.7, 121.8, 121.5, 107.4, 101.6, 56.2 (2 C). MS (ES^+) m/z 307 $[M+H]^+$. HRMS (ES^+) m/z calcd for $C_{18}H_{15}N_2O_3$ $[M+H]^+$ 307.1083, found 307.1079.

5.1.3.4. 8,9-Methylenedioxybenzo[c][1,8]phenanthroline-N-oxide. Yield 84%. IR 3079, 2909 (ν CH), 1701, 1618 (ν CNO), 1575, 1478 (ν CNO), 1444, 1256, 1158, 1044, 946 (OCH_2O), 844, 746 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 9.29 (s, 1H), 9.15 (d, 1H, J = 6.0 Hz), 8.93 (d, 1H, J = 2.0 Hz), 8.52 (d, 1H, J = 9.0 Hz), 8.42 (dd, 1H, J = 6.0, 2.0 Hz), 8.09 (s, 1H), 7.85 (d, 1H, J = 9.0 Hz), 7.22 (s, 1H), 6.24 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.3, 151.8, 149.2, 137.0, 136.3, 136.1, 132.7, 130.4, 130.3, 129.7, 128.2, 125.0, 123.3, 121.8, 105.4, 102.6, 100.0. MS (ES^+) m/z 291 $[M+H]^+$, 313 $[M+Na]^+$. HRMS (ES^+) m/z calcd for $C_{17}H_{11}N_2O_3$ $[M+H]^+$ 291.0770, found 291.0768.

5.1.4. General procedure for the chlorination of benzo[c] phenanthrolines-N-oxides

Benzo[*c*]phenanthroline-*N*-oxide dissolved in $POCl_3$ was heated at 160 °C. After the reaction was completed (TLC determination) the solution was cooled to rt and poured into ice. pH was adjusted to

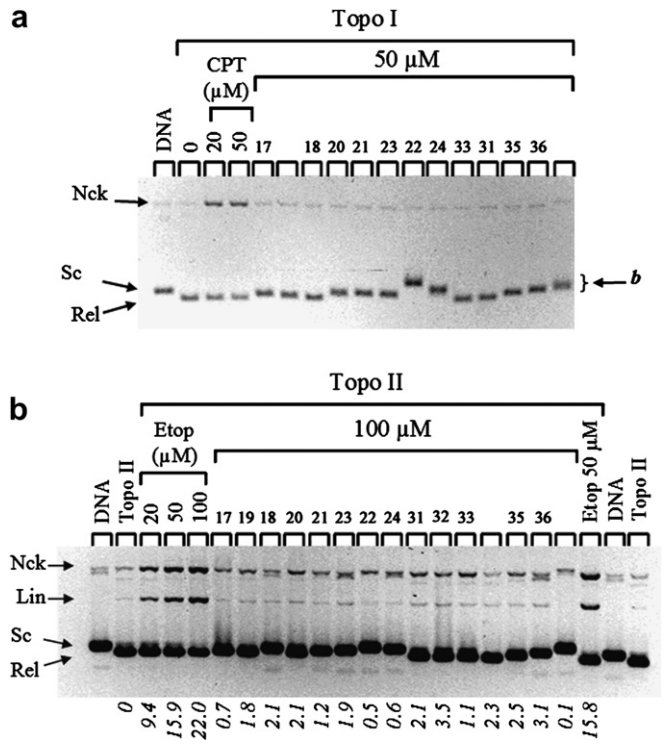


Fig. 5. Topoisomerase I and II inhibition. (a) Effect of benzo[c]phenanthrolines 17–36 on the relaxation of plasmid DNA by topoisomerase I at 50 μM compared to CPT at 20 and 50 μM. Native supercoiled pUC19 plasmid DNA (130 ng) (lane DNA) was incubated with 4 units topoisomerase I in the absence (lane 0) or presence of drug at the indicated concentration (50 μM). Reactions were stopped with sodium dodecylsulfate and treatment with proteinase K. DNA samples were separated by electrophoresis on 1% agarose gels containing ethidium bromide (1 μg/mL) and then photographed under UV light. Gel (b) shows the effect of benzo[c]phenanthrolines 17–36 on the relaxation of plasmid DNA by topoisomerase II at 100μM, compared to Etop at 20–100 μM. Native supercoiled pUC19 plasmid DNA (350 ng) (lane DNA) was incubated with 10 units topoisomerase I in the absence (lane TopoII) or presence of drug at the indicated concentration (100 μM). Reactions were stopped with sodium dodecylsulfate and treatment with proteinase K. DNA samples were separated by electrophoresis on 1% agarose gels containing ethidium bromide (1 μg/mL) and then photographed under UV light. Nck, nicked; Rel, relaxed; Sc, supercoiled DNA.

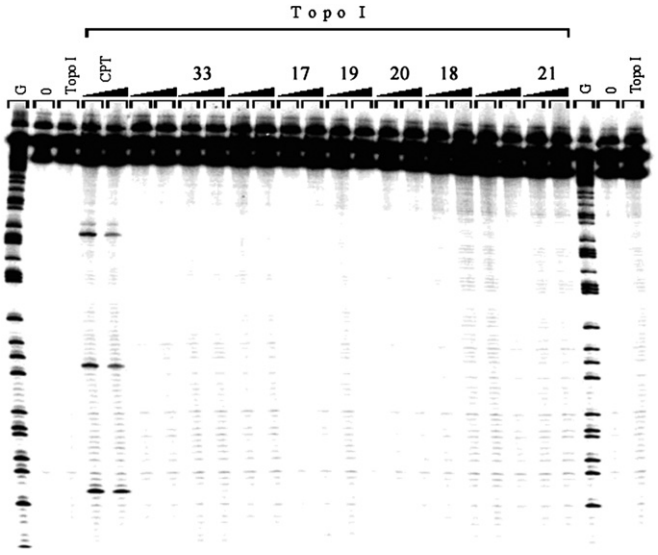


Fig. 6. Topoisomerase I poisoning. Cleavage of the 117-mer DNA fragment by topoisomerase I in the presence of the benzo[c]phenanthrolines **17**–**21** and 33. The 3′-end labeled fragment (0) was incubated in the absence (lane TopoI) or presence of the alkaloids at 10 or 50 μM. Camptothecin (CPT) was used at 20–50 μM. Topoisomerase I cleavage reactions were analyzed on a 8% denaturing polyacrylamide gel, compared to the guanine track labeled (G).

7–8 with an aqueous ammonia solution and the solution was left for 30 min. The precipitate formed was then filtered washed with water and dried. Purification by flash chromatography on silica gel (eluent CH_2Cl_2/MeOH 100:0 to 99.5:0.5) afforded the corresponding chloro compound as a white solid.

*5.1.4.1. 2-chloro-8,9-Dimethoxybenzo[c][1,7]phenanthroline (**15**).* Yield 80%. IR 3183 (ν CH), 1659, 1618, 1570, 1490, 1431, 1274, 1157, 1078, 821, 800, 738 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 9.58 (d, 1H, $J = 8.6$ Hz), 9.33 (s, 1H), 8.72 (d, 1H, $J = 9.3$ Hz), 8.18 (d, 1H, $J = 9.3$ Hz), 7.94 (s, 1H), 7.64 (d, 1H, $J = 8.6$ Hz), 7.46 (s, 1H), 4.21 (s, 3H), 4.16 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.4, 151.4, 151.0, 150.6, 148.1, 140.1, 136.1, 128.5, 127.2, 126.3, 124.8, 122.9, 122.7, 120.8, 107.3, 101.5, 56.2 (2 C). MS (ES+) *m/z* 325/327 $[M + H]^+$, 347/349 $[M + Na]^+$. HRMS (ES^+) *m/z* calcd for $C_{18}H_{14}N_2O_2Cl$ $[M + H]^+$ 325.0744, found 325.0739.

*5.1.4.2. 2-Chloro-8,9-methylenedioxybenzo[c][1,7]phenanthroline (**16**).* Yield 56%. IR 3033, 2094, 1655, 1605, 1578, 1473, 1442, 1306, 1253, 1197, 1122, 1038, 947, 910, 827 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 9.60 (d, 1H, $J = 8.6$ Hz), 9.28 (s, 1H), 8.65 (d, 1H, $J = 9.0$ Hz), 8.17 (d, 1H, $J = 9.0$ Hz), 7.99 (s, 1H), 7.65 (d, 1H, $J = 8.6$ Hz), 7.47 (s, 1H), 6.24 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.9, 151.5, 151.2, 150.9, 148.8, 142.3, 136.1, 130.5, 127.4, 127.2, 125.2, 125.0, 124.2, 122.8, 105.1, 102.1, 99.8. MS (ES+) *m/z* 309/311 $[M + H]^+$. HRMS (ES^+) *m/z* calcd for $C_{17}H_{10}N_2O_2Cl$ $[M + H]^+$ 309.0431, found 309.0427.

*5.1.4.3. 1-Chloro-8,9-dimethoxybenzo[c][1,8]phenanthroline (**29**).* Yield 82%. IR 2954 (ν CH), 2918, 2852, 1609, 1517 (ν CN), 1411, 1269, 1115, 987, 812 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 9.30 (s, 1H), 9.03 (d, 1H, $J = 5.5$ Hz), 8.53 (d, 1H, $J = 5.5$ Hz), 8.52 (d, 1H, $J = 9.2$ Hz), 8.36 (d, 1H, $J = 9.2$ Hz), 7.88 (s, 1H), 7.43 (s, 1H), 4.19 (s, 3H), 4.12 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6, 151.1, 150.9 (2 C), 143.4, 138.7, 135.7, 128.1, 126.0, 123.7, 123.5, 122.5, 117.6, 117.5, 107.4, 102.0, 56.2 (2 C). MS (ES+) *m/z* 325/327 $[M + H]^+$. HRMS (ES^+) *m/z* calcd for $C_{18}H_{14}N_2O_2Cl$ $[M + H]^+$ 325.0744, found 325.0741.

*5.1.4.4. 1-Chloro-8,9-methylenedioxybenzo[c][1,8]phenanthroline (**30**).* Yield 68%. IR 3134, 2925, 2860, 1640, 1610, 1266, 1210, 1103, 1036, 939, 840, 669 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 9.32 (s, 1H), 9.10 (d, 1H, $J = 6.0$ Hz), 8.58 (d, 1H, $J = 6.0$ Hz), 8.56 (d, 1H, $J = 9.0$ Hz), 8.45 (d, 1H, $J = 9.0$ Hz), 8.03 (s, 1H), 7.49 (s, 1H), 6.27 (s, 2H). MS (ES+) *m/z* 309/311 $[M + H]^+$. HRMS (ES^+) *m/z* calcd for $C_{17}H_{10}N_2O_2Cl$ $[M + H]^+$ 309.0431, found 309.0429.

5.1.5. General procedure for the preparation of dialkylamino alkylbenzo[c]phenanthroline

The chlorinated benzo[c]phenanthroline (70 mg, 0.241 mmol) was heated in a excess of the appropriate dialkylaminoalkylamine (7 ml) overnight. The reaction mixture was concentrated in vacuo and purified by chromatography on silica gel (eluent: CH_2Cl_2/MeOH/NH_4OH 100:0:0 to 90:10:0.5) to afford the corresponding compound.

*5.1.5.1. 2-[(2′-(Dimethylamino)ethyl)amino]-8,9-dimethoxybenzo[c][1,7]phenanthroline (**17**).* Yield 38%. IR 3366 (ν NH), 2930 (ν CH), 2655, 2637, 1616 (δ NH), 1587, 1522, 1497, 1458, 1270 (ν CN), 1155, 1038, 822 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 9.19 (d, 1H, $J = 9.0$ Hz), 9.16 (s, 1H), 8.44 (d, 1H, $J = 9.2$ Hz), 7.88 (d, 1H, $J = 9.2$ Hz), 7.78 (s, 1H), 7.29 (s, 1H), 6.84 (d, 1H, $J = 9.0$ Hz), 4.77 (bs, NH), 4.11 (s, 3H), 4.04 (s, 3H), 3.62 (t, 2H, $J = 6.0$ Hz), 2.62 (t, 2H, $J = 6.0$ Hz), 2.30 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.8, 152.9, 150.2, 149.0, 148.2,

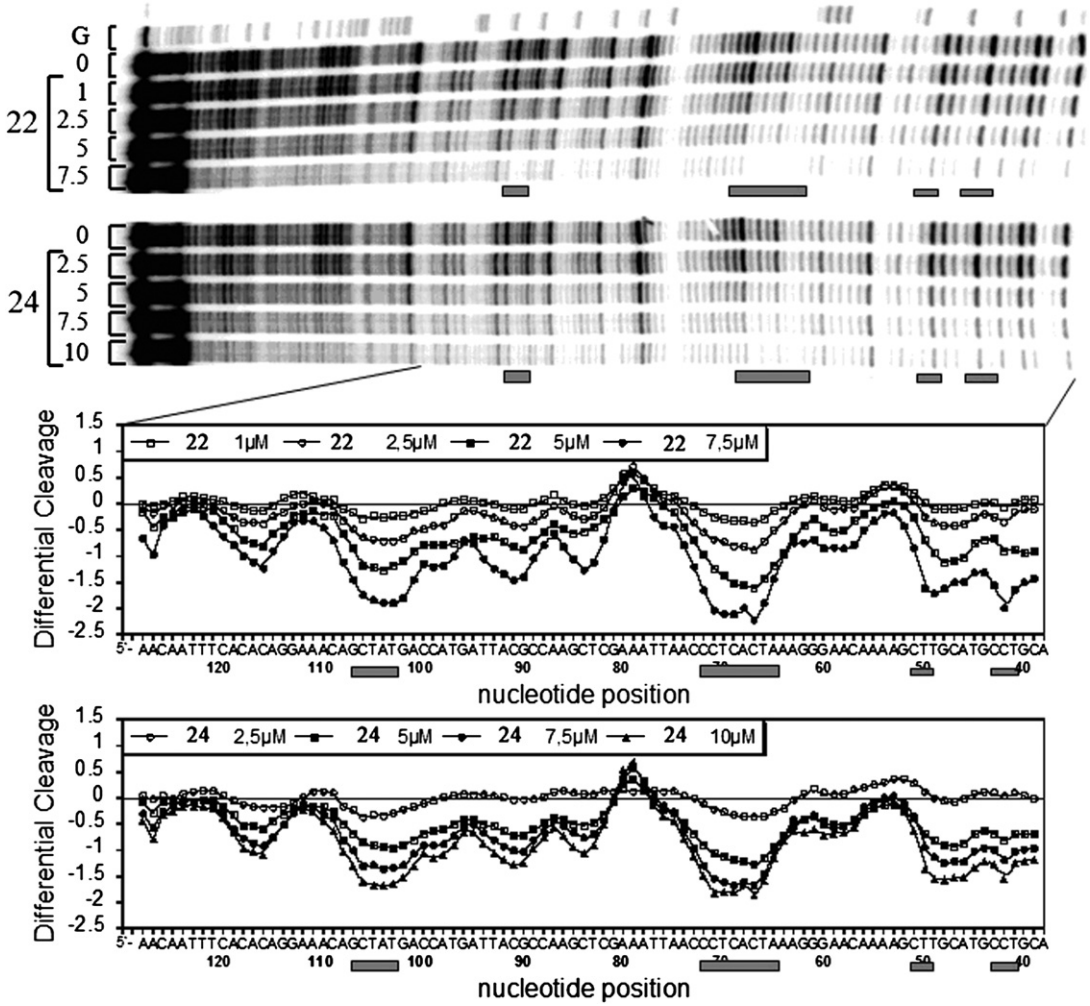


Fig. 7. Sequence-specific DNA binding. Appropriate concentrations of the various benzophenanthrolines were incubated with the 265 bp 3′-end-radio-labeled DNA fragment prior to DNA digestion using DNase I (0.01 unit/ml). The digestion reaction was stopped by freeze drying and lyophilization of the samples. DNA samples were separated by electrophoresis on an 8% denaturing polyacrylamide gel. Each resolved band on the autoradiograph was assigned to a particular bond within the DNA fragment from comparison with its position relative to the guanines sequencing standard (G-track).

140.7, 133.7, 128.8, 126.3, 123.0, 121.5, 120.6, 118.0, 111.3, 107.1, 101.1, 58.1, 56.0 (2C), 45.1 (2 C), 38.8. MS (DIC/NH_3) *m/z* 376 $[M]^+$. HRMS (ES^+) *m/z* calcd for $C_{22}H_{25}N_4O_2$ $[M+H]^+$ 376.1978, found 376.1974.

*5.1.5.2. 2-[(3′-(Dimethylamino)propyl)amino]-8,9-dimethoxybenzo [c][1,7]phenanthroline (**18**).* Yield 19%. IR 3328, 2922, 1681, 1654, 1615, 1541, 1457, 1258, 1149, 1036, 825, 765, 740 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 9.23 (d, 1H, $J = 8.0$ Hz), 9.21 (s, 1H), 8.49 (d, 1H, $J = 9.0$ Hz), 7.89 (d, 1H, $J = 9.0$ Hz), 7.85 (s, 1H), 7.35 (s, 1H), 6.85 (d, 1H, $J = 8.0$ Hz), 5.85 (bs, NH), 4.15 (s, 3H), 4.08 (s, 3H), 3.63 (t, 2H, $J = 6.0$ Hz), 2.51 (t, 2H, $J = 6.0$ Hz), 2.31 (s, 6H), 1.89 (quint, 2H, $J = 6.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.0, 153.0, 150.3, 149.4, 148.7, 141.1, 133.9, 128.9, 126.5, 123.1, 121.7, 120.9, 118.0, 110.8, 107.2, 101.3, 58.2, 56.1 (2 C), 45.5 (2 C), 41.0, 26.9. MS (ES^+) *m/z* 391 $[M+H]^+$. HRMS (ES^+) *m/z* calcd for $C_{23}H_{27}N_4O_2$, $[M+H]^+$ 391.2134, found 391.2130.

*5.1.5.3. 2-[(2′-(Diethylamino)ethyl)amino]-8,9-dimethoxybenzo[c] [1,7]phenanthroline (**19**).* Yield 41%. IR 3355 (ν NH), 2922 (ν CH), 2660, 1653, 1570, 1520, 1418, 1259, 1205, 1077, 819 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 9.23 (d, 1H, $J = 8.0$ Hz), 9.22 (s, 1H), 8.50 (d, 1H, $J = 9.0$ Hz), 7.90 (d, 1H, $J = 9.0$ Hz), 7.86 (s, 1H), 7.36 (s, 1H), 6.86 (d, 1H, $J = 8.0$ Hz), 5.79 (bs, NH), 4.18 (s, 3H), 4.11 (s, 3H), 3.66 (q, 2H, $J = 6.0$ Hz), 2.85 (t, 2H, $J = 6.0$ Hz), 2.68 (q, 4H, $J = 7.0$ Hz), 1.12 (t, 6H, $J = 7.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.9, 152.9, 150.3, 149.3, 148.4, 140.9, 133.7, 128.8, 126.6, 123.0, 121.6, 120.7, 117.9, 111.2, 107.2, 101.2, 56.1, 53.4 (2C), 46.6 (2 C), 39.0, 11.6 (2 C). MS (DIC/NH_3) *m/z* 404 $[M]^+$. HRMS (ES^+) *m/z* calcd for $C_{24}H_{29}N_4O_2$ $[M+H]^+$ 405.2291, found 405.2286.

*5.1.5.4. 2-[(3′-(Diethylamino)propyl)amino]-8,9-dimethoxybenzo[c] [1,7]phenanthroline (**20**).* Yield 42%. IR 3355 (ν NH), 3053, 2969 (ν CH), 1615 (δ NH), 1537, 1471, 1385, 1271 (ν CN), 1224, 1150, 824, 732, 707 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 9.16 (d, 1H, $J = 8.0$ Hz), 9.14 (s, 1H), 8.42 (d, 1H, $J = 9.0$ Hz), 7.85 (d, 1H, $J = 9.0$ Hz), 7.76 (s, 1H), 7.28 (s, 1H), 6.81 (d, 1H, $J = 8.0$ Hz), 6.31 (bs, NH), 4.10 (s, 3H), 4.01 (s, 3H), 3.64 (t, 2H, $J = 6.0$ Hz), 2.63 (q, 4H, $J = 7.0$ Hz), 2.52 (t, 2H, $J = 6.0$ Hz), 1.89 (quint, 2H), 1.12 (t, 6H, $J = 7.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.9, 152.9, 150.3, 149.3, 148.4, 140.8, 133.6, 128.8, 126.4, 122.9, 121.6, 120.6, 117.9, 111.3, 107.1, 101.2, 56.0 (2C), 51.7, 46.7 (2 C), 41.1, 25.7, 11.2 (2 C). MS (DIC/NH_3) *m/z* 418 $[M]^+$. HRMS (ES^+) *m/z* calcd for $C_{25}H_{31}N_4O_2$ $[M+H]^+$ 419.2447, found 419.2445.

*5.1.5.5. 2-[(2′-(Dimethylamino)ethyl)amino]-8,9-methylenedioxybenzo [c][1,7]phenanthroline (**21**).* Yield 27%. IR 3407 (ν NH), 2923 (ν CH), 1665, 1545, 1473, 1400, 1364, 1257 (ν CN), 1101, 1037, 821, 750 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 9.20 (d, 1H, $J = 8.0$ Hz), 9.12 (s, 1H), 8.38

(d, 1H, $J = 9.0$ Hz), 7.86 (d, 1H, $J = 9.0$ Hz), 7.84 (s, 1H), 7.31 (s, 1H), 6.84 (d, 1H, $J = 8.0$ Hz), 6.72 (bs, NH), 6.12 (s, 2H), 3.63 (q, 2H, $J = 6.0$ Hz), 2.42 (t, 2H, $J = 6.0$ Hz), 2.22 (t, 6H, $J = 6.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.8, 151.6, 150.5, 148.2, 147.6, 141.0, 133.8, 130.9, 126.4, 123.3, 122.9, 120.5, 118.7, 111.4, 104.9, 101.8, 99.4, 57.8, 45.0 (2 C), 41.0. MS (ES^+) m/z 361 $[M + H]^+$. HRMS (ES^+) m/z calcd for $C_{21}H_{21}N_4O_2$, $[M + H]^+$ 361.1665, found 361.1661.

*5.1.5.6. 2-[(3′-(Dimethylamino)propyl)amino]-8,9-methylenedioxybenzo[c][1,7]phenanthroline (**22**).* Yield 31%. IR 3345 (ν NH), 2923 (ν CH), 2853, 1612, 1544, 1470, 1254 (ν CN), 1038, 825, 725 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.21 (d, 1H, $J = 8.0$ Hz), 9.12 (s, 1H), 8.39 (d, 1H, $J = 9.0$ Hz), 7.86 (d, 1H, $J = 9.0$ Hz), 7.84 (s, 1H), 7.31 (s, 1H), 6.83 (d, 1H, $J = 8.0$ Hz), 6.13 (s, 2H), 5.74 (bs, NH), 3.59 (t, 2H, $J = 6.0$ Hz), 2.48 (t, 2H, $J = 6.0$ Hz), 2.29 (s, 6H), 1.86 (quint, 2H, $J = 6.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.1, 151.5, 150.5, 148.4, 147.6, 141.1, 134.0, 130.9, 126.5, 123.3, 122.9, 120.5, 118.6, 110.7, 104.9, 101.7, 99.4, 57.9, 45.0 (2 C), 40.7, 26.9. MS (ES^+) m/z 375 $[M + H]^+$, 397 $[M + Na]^+$. HRMS (ES^+) m/z calcd for $C_{22}H_{23}N_4O_2$, $[M + H]^+$ 375.1821, found 375.1815.

*5.1.5.7. 2-[(2′-(Diethylamino)ethyl)amino]-8,9-methylenedioxybenzo[c][1,7]phenanthroline (**23**).* Yield 16%. IR 3384 (ν NH), 2924 (ν CH), 2870, 1654, 1541, 1457, 1259 (ν CN), 1116, 1038, 936 (OCH_2O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (d, 1H, $J = 8.0$ Hz), 9.18 (s, 1H), 8.44 (d, 1H, $J = 9.0$ Hz), 7.91 (s, 1H), 7.88 (d, 1H, $J = 9.0$ Hz), 7.37 (s, 1H), 6.90 (d, 1H, $J = 8.0$ Hz), 6.17 (s, 2H), 5.88 (bs, NH), 3.70 (q, 2H, $J = 6.0$ Hz), 2.90 (t, 2H, $J = 6.0$ Hz), 2.73 (q, 4H, $J = 7.0$ Hz), 1.19 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.1, 151.6, 150.0, 148.6, 147.5, 141.3, 133.8, 131.1, 126.4, 123.5, 123.2, 120.5, 117.0, 110.9, 104.9, 102.0, 99.2, 58.1, 46.6 (2 C), 39.0, 11.6 (2 C). MS (ES^+) m/z 389 $[M + H]^+$. HRMS (ES^+) m/z calcd for $C_{23}H_{24}N_4O_2$, $[M + H]^+$ 389.1978, found 389.1975.

*5.1.5.8. 2-[(3′-(Diethylamino)propyl)amino]-8,9-methylenedioxybenzo[c][1,7]phenanthroline (**24**).* Yield 8%. IR 3317 (ν NH), 2924 (ν CH), 2853, 1613, 1544, 1451, 1254 (ν CN), 1226, 1192, 1038, 826, 745 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.21 (d, 1H, $J = 8.0$ Hz), 9.16 (s, 1H), 8.43 (d, 1H, $J = 9.0$ Hz), 7.89 (s, 1H), 7.87 (d, 1H, $J = 9.0$ Hz), 7.35 (s, 1H), 6.82 (d, 1H, $J = 8.0$ Hz), 6.20 (bs, NH), 6.16 (s, 2H), 3.66 (t, 2H, $J = 6.0$ Hz), 2.71 (t, 2H, J = 6.0 Hz), 2.66 (q, 4H, $J = 7.0$ Hz), 1.92 (quint, 2H, $J = 6.0$ Hz), 1.08 (t, 6H, $J = 7.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.1, 151.5, 150.5, 148.6, 147.5, 141.2, 133.8, 130.9, 126.7, 123.2, 122.9, 120.6, 118.5, 111.2, 105.0, 101.7, 99.4, 53.4, 46.8 (2 C), 41.3, 25.9, 11.4 (2 C). MS (ES^+) m/z 403 $[M + H]^+$. HRMS (ES^+) m/z calcd for $C_{24}H_{27}N_4O_2$, $[M + H]^+$ 403.2134, found 403.2130.

*5.1.5.9. 1-[(2′-(Dimethylamino)ethyl)amino]-8,9-dimethoxybenzo[c][1,8]phenanthroline (**31**).* Yield 52%. IR 3370 (ν NH), 2946 (ν CH), 1666, 1519, 1386, 1270 (ν CN), 1038, 830, 782 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.42 (d, 1H, $J = 9.0$ Hz), 8.37 (d, 1H, $J = 5.0$ Hz), 8.32 (d, 1H, $J = 5.0$ Hz), 8.02 (d, 1H, $J = 9.0$ Hz), 7.94 (s, 1H), 7.46 (s, 1H), 6.17 (bs, NH), 4.20 (s, 3H), 4.13 (s, 3H), 3.73 (q, 2H, $J = 6.0$ Hz), 2.74 (t, 2H, $J = 6.0$ Hz), 2.38 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.4, 153.1, 150.5, 150.4, 143.2, 139.6, 137.2, 128.1, 123.0 (2C), 120.4, 119.3, 116.4, 107.3, 107.2, 101.8, 58.1, 56.2 (2C), 45.1 (2 C), 39.2. MS (ES^+) m/z 377 $[M + H]^+$. HRMS (ES^+) m/z calcd for $C_{22}H_{25}N_4O_2$, $[M + H]^+$ 377.1978, found 377.1975.

*5.1.5.10. 1-[(3′-(Dimethylamino)propyl)amino]-8,9-dimethoxybenzo[c][1,8]phenanthroline (**32**).* Yield 50%. IR 3371 (ν NH), 2939 (ν CH), 1615 (δ NH), 1584, 1484, 1416, 1270 (ν CN), 1164, 1070, 827, 788 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.30 (d, 1H, $J = 9.0$ Hz), 8.28–8.24 (m, 2H), 7.84 (s, 1H), 7.81 (d, 1H, $J = 9.0$ Hz), 7.66 (bs, NH), 7.37 (s, 1H), 4.15 (s, 3H), 4.08 (s, 3H), 3.74 (t, 2H, $J = 6.0$ Hz), 2.59 (t, 2H, $J = 6.0$ Hz), 2.39 (s, 6H), 1.40 (quint, 2H, $J = 6.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.8, 152.9 (2C), 150.3, 143.5, 139.6, 137.1, 128.1, 122.9, 122.8, 120.3, 119.1, 116.5, 107.2, 106.4, 101.7, 59.6, 56.1(2C), 45.5 (2 C), 45.5, 25.3. MS (DIC/NH_3) m/z 390 $[M]^+$. HRMS (ES^+) m/z calcd for $C_{23}H_{27}N_4O_2$, $[M + H]^+$ 391.2134, found 391.2131.

*5.1.5.11. 1-[(2′-(Diethylamino)ethyl)amino]-8,9-dimethoxybenzo[c][1,8]phenanthroline (**33**).* Yield 46%. IR 3373 (ν NH), 2969 (ν CH), 1669, 1615 (δ NH), 1518, 1483, 1417, 1271 (ν CN), 1164, 1068, 827, 788 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.43 (d, 1H, $J = 9.0$ Hz), 8.34 (d, 1H, $J = 5.0$ Hz), 8.28 (d, 1H, $J = 5.0$ Hz), 8.03 (d, 1H, $J = 9.0$ Hz), 7.91 (s, 1H), 7.41 (s, 1H), 6.56 (bs, NH), 4.18 (s, 3H), 4.11 (s, 3H), 3.72 (t, 2H, $J = 6.0$ Hz), 2.90 (t, 2H, $J = 6.0$ Hz), 2.71 (q, 4H, $J = 7.0$ Hz), 1.14 (t, 6H, $J = 7.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.3, 153.8, 150.3 (2 C), 143.1, 139.9, 137.2, 128.2, 123.0 (2C), 120.4, 119.5, 116.5, 107.1 (2C), 101.8, 56.1 (2C), 51.5, 46.9 (2 C), 39.2, 11.2 (2 C). MS (DIC/NH_3) m/z 404 $[M]^+$. HRMS (ES^+) m/z calcd for $C_{24}H_{29}N_4O_2$, $[M + H]^+$ 405.2291, found 405.2289.

*5.1.5.12. 1-[(3′-(Diethylamino)propyl)amino]-8,9-dimethoxybenzo[c][1,8]phenanthroline (**34**).* Yield 41%. IR 3341 (ν NH), 2968 (ν CH), 1614 (δ NH), 1584, 1518, 1484, 1416, 1337, 1270 (ν CN), 1164, 1071, 827, 746 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (s, 1H), 8.30 (d, 1H, $J = 9.0$ Hz), 8.28 (d, 1H, $J = 5.0$ Hz), 8.25 (d, 1H, $J = 5.0$ Hz), 7.99 (bs, NH), 7.92 (d, 1H, $J = 9.0$ Hz), 7.87 (s, 1H), 7.38 (s, 1H), 4.17 (s, 3H), 4.09 (s, 3H), 3.74 (q, 2H, $J = 6.0$ Hz), 2.72 (t, 2H, $J = 6.0$ Hz), 2.69 (q, 4H, $J = 7.0$ Hz), 1.94 (quint, 2H, $J = 6.0$ Hz), 1.15 (t, 6H, $J = 7.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.9, 153.0 (2C), 150.3, 143.5, 139.7, 137.2, 128.2, 123.0 (2C), 120.9, 119.0, 116.6, 107.3, 107.6, 101.9, 56.3 (2 C), 51.2, 46.9 (2 C), 45.0, 24.8, 11.2 (2 C). MS (ES^+) m/z 419 $[M + H]^+$. HRMS (ES^+) m/z calcd for $C_{25}H_{31}N_4O_2$, $[M + H]^+$ 419.2447, found 419.2447.

*5.1.5.13. 1-[(2′-(Dimethylamino)ethyl)amino]-8,9-methylenedioxybenzo[c][1,8]phenanthroline (**35**).* Yield 17%. IR 3413 (ν NH), 2951 (ν CH), 2858, 1655, 1623 (δ NH), 1585, 1509, 1466, 1267 (ν CN), 1197, 1047, 951 (OCH_2O), 845, 738 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H), 8.28 (d, 1H, $J = 9.0$ Hz), 8.25 (d, 1H, $J = 6.0$ Hz), 8.19 (d, 1H, $J = 6.0$ Hz), 8.08 (d, 1H, $J = 9.0$ Hz), 7.88 (s, 1H), 7.36 (s, 1H), 6.51 (bs, NH), 6.17 (s, 2H) 3.70 (t, 2H, $J = 6.0$ Hz), 2.60 (t, 2H, $J = 6.0$ Hz), 2.38 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.3, 151.7, 150.5, 148.7, 143.2, 139.5, 137.0, 130.2, 124.3, 123.6, 120.6, 119.6, 116.6, 107.1, 105.0, 102.0, 100.0, 58.1, 45.0 (2 C), 38.8. MS (ES^+) $m/z = 361$ $[M + H]^+$. HRMS (ES^+) m/z calcd for $C_{21}H_{21}N_4O_2$, $[M + H]^+$ 361.1665, found 361.1663.

*5.1.5.14. 1-[(3′-(Dimethylamino)propyl)amino]-8,9-methylenedioxybenzo[c][1,8]phenanthroline (**36**).* Yield 6%. IR 3453 (ν NH), 2925 (ν CH), 1635 (δ NH), 1533, 1493, 1471, 1262 (ν CN), 1118, 1045, 946 (OCH_2O), 843, 739 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.30–8.26 (m, 2H), 8.26 (d, 1H, $J = 9.0$ Hz), 7.90 (s, 1H), 7.82 (d, 1H, $J = 9.0$ Hz), 7.72 (bs, NH), 7.38 (s, 1H), 6.19 (s, 2H), 3.75 (t, 2H, $J = 6.0$ Hz), 2.51 (t, 2H, $J = 6.0$ Hz), 2.21 (s, 6H), 1.95 (quint, 2H, $J = 6.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.8, 151.5, 150.4, 148.5, 143.6, 140.0, 137.1, 130.2, 124.3, 123.3, 120.4, 119.3, 116.5, 106.5, 105.0, 102.0, 99.9, 59.6, 45.5 (2 C), 42.8, 25.4. MS (ES^+) m/z 375 $[M + H]^+$. HRMS (ES^+) m/z calcd for $C_{22}H_{21}N_4O_2$, $[M + H]^+$ 375.1821, found 375.1818.

*5.1.5.15. 1-[(3′-(Diethylamino)propyl)amino]-8,9-methylenedioxybenzo[c][1,8]phenanthroline (**37**).* Yield 9%. IR 3373 (ν NH), 2923 (ν CH), 2853, 1647, 1617 (δ NH), 1540, 1471, 1375, 1261 (ν CN), 1037, 942 (OCH_2O), 828 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.31 (d, 1H, $J = 9.0$ Hz), 8.29 (d, 1H, $J = 6.0$ Hz), 8.19 (d, 1H, $J = 6.0$ Hz), 8.02 (d, 1H, $J = 9.0$ Hz), 7.97 (s, 1H), 7.42 (s, 1H), 6.35 (bs, NH), 6.21 (s, 2H),

3.77 (t, 2H, $J = 6.0$ Hz), 2.76 (q, 4H), 1.99 (t, 2H, $J = 6.0$ Hz) 1.77–1.72 (m, 2H), 1.19 (t, 6H). MS (ES^+) m/z 403 $[M + H]^+$. HRMS (ES^+) m/z calcd for $C_{24}H_{27}N_4O_2$, $[M + H]^+$ 403.2134, found 403.2132.

5.2. Biology

5.2.1. Cytotoxicity (Table 1)

Murine leukemia L1210 cells from the American Type Culture Collection (Rockville Pike, MD) were grown in RPMI medium 1640 supplemented with 10% foetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 lg/mL streptomycin and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured by microculture tetrazolium assay essentially as described. Cells were exposed for 48 h to nine graded concentrations in triplicate of the test drug. Results are expressed as IC50 (mean, n 1/43), which is defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells.

5.2.2. UV–visible absorption spectrometry (Fig. 1)

CT-DNA were obtained from Sigma. After dissolution, CT-DNA was deproteinized using sodium dodecyl sulfate and then dialyzed against 1 mM sodium cacodylate (pH 7.0). The concentrations of DNA were determined from their molar extinction coefficients of 6600 $M^{-1}\,cm^{-1}$. Tested compounds, as well as the reference molecules camptothecin and etoposide (Sigma), were dissolved in DMSO as 10 mM solutions. Further dilutions were made in the appropriate aqueous binding or reaction buffers.

UV–visible spectrum were measured using an Uvikon 943 spectrophotometer. Samples containing 20 μM of the tested drugs were prepared in 1 mL of BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, pH 7.1) in the presence or absence of 20 μM of CT-DNA and transferred in a quartz cuvette of 10 mm pathlength. The various spectra were recorded from 230 nm to 500 nm and are referenced against a cuvette containing the same DNA concentration in the same buffer.

5.2.3. Melting temperature studies (Table 2)

For melting temperature measurements, 20 μM of CT-DNA were incubated alone (control Tm) or with increasing concentrations of the various benzophenanthroline derivatives 1 mL of BPE buffer (base pair/drug ratio of 1). The absorbency at 260 nm was measured in quartz cells every min over the range of 20–100 °C with an increment of 1 °C per min using an Uvikon XL spectrophotometer coupled with a peltier temperature controller. The T_m values were deduced from the midpoint of the hyperchromic transition of double strand to single strand DNA.

5.2.4. Fluorescence spectroscopy (Fig. 2)

Fluorescence spectral measurement were recorded using 20 μM of the fluorescent drugs incubated in 1 mL of BPE buffer in the presence or absence of 200 μM of CT-DNA in a quartz cuvette of 10 mm pathlength using a SPEX Fluorolog spectrofluorimeter (Jobin Ivon, Longjumeau, France). The excitation wavelengths were 268 nm for compound **35**, 275 nm for compounds **31**, **32**, **33** and **36**, and 285 nm for all other evaluated compounds. The emission spectra were scanned at the wavelength specified in the abscissa of the figures.

5.2.5. Circular dichroism (Fig. 3)

The CD spectra from samples containing 50 μM of the various drugs incubated in 1 mL of sodium cacodylate (1 mM, pH 7.0) without (control) or with 200 μM (base pairs) of CT-DNA were collected in a quartz cell of 10 mm path length using a J-810 Jasco spectropolarimeter at a fixed 20 °C temperature controlled by a PTC-424S/L peltier type cell changer (Jasco). The spectra were recorded from 500 to 230 nm with a resolution of 0.1 nm.

5.2.6. Topoisomerase I-mediated DNA relaxation and DNA cleavage assays (Figs. 4 and 5a)

The experiments were conducted as presented elsewhere [20]. Briefly, supercoiled pUC19 plasmid DNA (130 ng) was incubated with 4 units of human recombinant topoisomerase I (TopoGen) at 37 °C for 45 min in 20 μL of relaxation buffer (50 mM Tris pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, 1 mM ATP) in the presence of graded concentrations (from 0.5 to 20 μM) of the tested compounds. The reactions were stopped by the addition of SDS (0.25%) and proteinase K (250 μg/mL) followed by an incubation of 30 min at 50 °C. Then 3 μL of the electrophoresis dye mixture was added to each DNA samples and loaded on a 1% agarose gel containing (topoisomerase DNA cleavage gel) or not (inhibition of the relaxation of DNA) ethidium bromide (1 μg/mL) at room temperature for 2 h at 120 V. Gels run without ethidium bromide were then stained post-electrophoresis using an ethidium bromide-containing bath. Both gels were washed prior to be photographed under UV light.

5.2.7. Topoisomerase II cleavage assay (Fig. 5b)

Supercoiled pUC19 plasmid DNA (350 ng) was incubated with 50 μM of the various benzophenanthroline derivatives or etoposide, as control, (20 or 50 μM) prior to the addition of 10 units of human topoisomerase II (TopoGen) at 37 °C for 45 min in the appropriate cleavage buffer. SDS (0.25%) and proteinase K (250 μg/mL) were then added to stop the reaction during 30 min at 50 °C. DNA samples were loaded on 1% agarose gels containing ethidium bromide for 2 h at 120 V in TBE buffer. After migration, gels were washed and photographed under UV light.

5.2.8. DNase I footprinting (Fig. 7)

Experiments were performed as previously described and using a 265 bp 3′-end-labelled DNA fragment obtained from double digestion of the pBS plasmid (Stratagene, La Jolla, CA) at *Eco*RI and *Pvu*II restriction sites followed by radiolabelling of one strand by incorporation of α- [^{32}P]-dATP (3000 Ci/mmol, GE Healthcare, Buckinghamshire, England) at the *Eco*RI restriction site using AMV reverse transcriptase (Ozyme, France). Appropriate concentrations of the various benzophenanthrolines were incubated with the 265-bp radio-labeled DNA fragment for 15 min at 37 °C to ensure equilibrium prior to DNA digestion using DNase I (0.01 unit/ml) in 20 mM NaCl, 2 mM $MgCl_2$, 2 mM $MnCl_2$ (pH 7.3), for 3 min. The digestion reaction was stopped by freeze drying and lyophilization of the samples. The DNA pellets were dissolved in 5 μL of denaturing loading buffer (80% formamide solution containing tracking dyes), heated at 90 °C for 4 min and chilled in ice for another 4 min prior to be loaded on a 8% denaturing polyacrylamide gel for 90 min at 65 W in TBE buffer. The data were collected using a Molecular Dynamics STORM 860 PhosphorImager and analyzed using the ImageQuant version 4.1 software. Each resolved band on the autoradiograph was assigned to a particular bond within the DNA fragment from comparison with its position relative to the guanines sequencing standard (G-track) classically obtained using DMS and piperidine treatment of the same DNA fragment.

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