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Synthesis and cytotoxic activity of benzo[*c*][1,7] and [1,8]phenanthrolines analogues of nitidine and fagaronine

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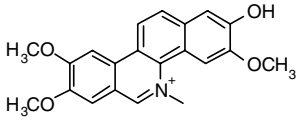
Abstract—Fagaronine and nitidine are natural benzo[*c*] phenanthridinium alkaloids, which display antileukemic activity. Both act as topoisomerase I and topoisomerase II inhibitors. The objective of the present study was to prepare noncharged isosters of these compounds, with replacement of the aromatic A ring by a pyridine ring, present in other topoisomerase I inhibitors. Various 7,8- and 8,9-dimethoxy and metylenedioxy benzo[*c*][1,7] and [1,8]phenanthrolines were readily synthesized by benzyne-mediated cyclization of the corresponding substituted *N*-(2-halobenzyl)-5-quinolinamines or 5-isoquinolinamines. In both series, compounds bearing oxygenated substituents at positions 8 and 9 exhibited cytotoxic properties towards L1210 murine leukemia cells, which may result from their capacities to intercalate into DNA. Topoisomerase I inhibition was observed for all active compounds.

1. Introduction

DNA topoisomerases are ubiquitous enzymes that catalyze the breaking and rejoining of DNA strands. They are involved in the regulation of the topological states of DNA, resolving the topological problems that arise during several essential nuclear processes, including transcription, recombination, and replication in the course of cell division.[1,2] Two major types of topoisomerases occur in eukaryotic cells: type I and type II, depending on their mechanism of action, which implies either a single or a double-stranded DNA transient breakage. Both enzymes represent important targets for the development of new antitumor drugs.[3] Indeed, the biological activity of several anticancer agents appears to be correlated with their ability to stabilize enzyme–DNA cleavable complexes.[4] Topotecan and irinotecan, derived from the structure of the natural alkaloid camptothecin, are topoisomerase I poisons, which were launched in 1996 and 1994, respectively.[5,6] Anthracyclines, exemplified by daunorubicin and doxorubicin, are typical examples of topoisomerase II inhibitors currently used in cancer chemotherapy.[7,8]

It is interesting to note that most of the anticancer agents, which interfere with topoisomerases were initially developed from a natural product, isolated either from a higher plant or from a microorganism. In this context, benzo[*c*]phenanthridinium alkaloids, illustrated by fagaronine (**1**) and nitidine (**2**), both isolated from trees belonging to the genus *Zanthoxylum* (Rutaceae), appear as interesting models for the development of new antitumor drugs.[9–11] Indeed, these compounds have



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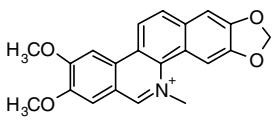

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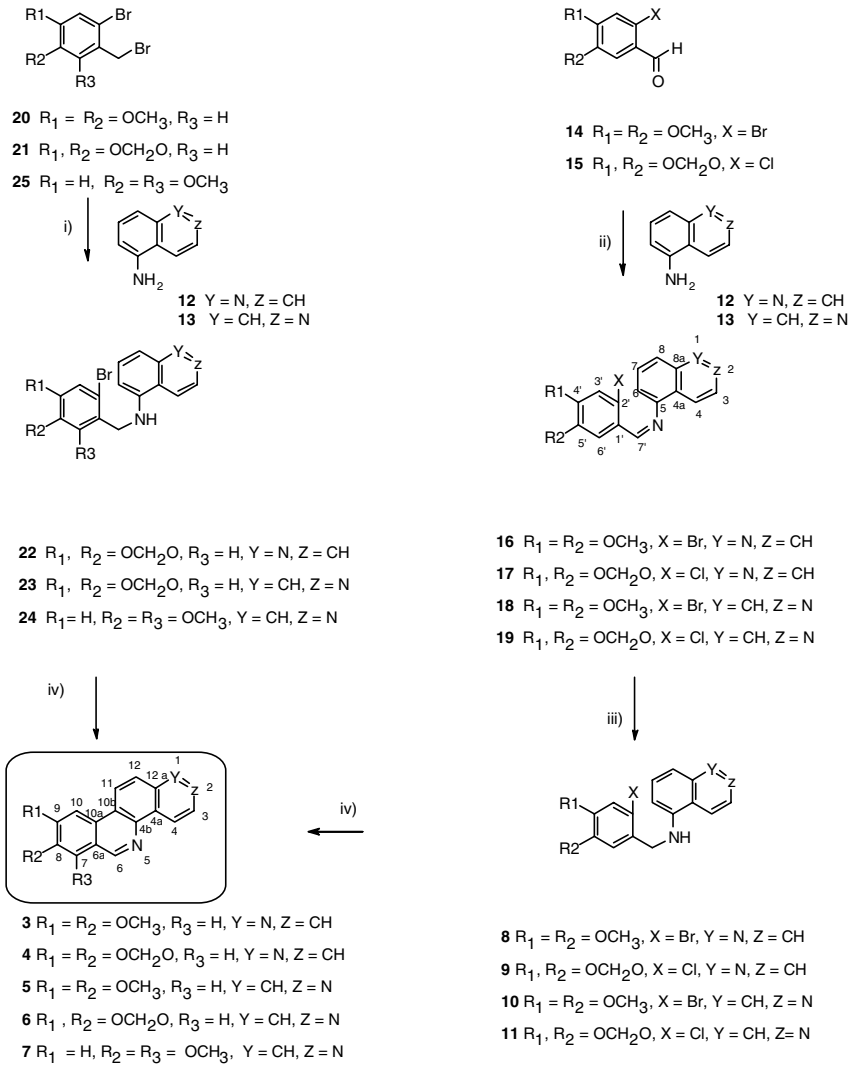
been shown to be cytotoxic and to act as topoisomerase I inhibitors at low concentrations and topoisomerase II inhibitors at higher concentrations.[12–14] Preclinical in vivo experiments conducted by the NCI. revealed the antileukemic activity of fagaronine in rodents.[15] Nevertheless, these trials were rapidly discontinued, due to several points, which appeared as severe drawbacks for future development, including a quaternary structure limiting the intracellular penetration of the drug and a narrow spectrum of action, restricted to leukemias. The imminium charge of these alkaloids had been first speculated as necessary for biological activity, in connection with its reactivity towards nucleophilic attack. Later on, Bisagni and Janin demonstrated that noncharged analogues of nitidine could act as topoisomerase poisons and exhibited potent cytotoxic activity.[16] Based on this finding, the group of LaVoie recently developed promising structural analogues in the benzo[*i*]phenanthridine series bearing four oxygenated substituents at positions 2, 3, 8 and 9, in the form of methoxy or methylenedioxy groups in reference to the natural models.[17–19] Interestingly, in the related 11*H*-indeno[1,2-*c*] isoquinoline-5,11-dione and 5*H*-dibenzo [*c*,*h*][1,6] naphthyridine-6-one series, several compounds bearing only two *ortho*-oxygenated substituents also revealed potent cytotoxic and topoisomerase I inhibiting activities, some of them being effective in regressing tumor growth in vivo.[20–22] In this context, the replacement of the aromatic A ring of nonquaternary benzo[*c*]phenanthridines by a pyridine ring, present in some other topoisomerase I inhibitors such as intoplicine,[23,24] appeared to us as an interesting pharmacomodulation in this series of natural alkaloids analogues. We describe here the synthesis and biological activities of several 7,8- and 8,9-dimethoxy and methylenedioxy benzo[*c*][1,7] and [1,8]phenanthrolines.

2. Results and discussion

Scheme 1 outlines the synthetic procedures used for the preparation of benzo[*c*][1,7]phenanthrolines **3** and **4**, and benzo[*c*][1,8]phenanthrolines **5**–**7**. The key step of this approach was a benzyne-mediated cyclization of a



Scheme 1. Synthetic approach to the preparation of benzo [*c*][1,7] and [1,8] phenanthrolines derivates. (i) neutral, alumina, toluene reflux , (ii) toluene reflux; (iii) $NaBH_4$, MeOH, 0 °C; (iv) (1) LDA, THF, −78 °C; (2) air oxidation.

conveniently substituted *N*-(2-halobenzyl)-5-quinolinamine or 5-isoquinolinamine. Lithium diisopropylamide was used for this purpose, since this reagent had previously given satisfactory results for the cyclization of various anils into benzo[*c*]phenanthridines[25] and heterocyclic structural analogues, such as pyranophenanthridines,[26] bearing methoxy and methylenedioxy groups.

The required *N*-(2-halobenzyl)-5-quinolinamines and isoquinolinamines **8**–**11**, with oxygenated substituents at positions 4 and 5 on the benzyl moiety, were prepared by condensation of 5-aminoquinoline (**12**) and 5-aminoisoquinoline (**13**) either with 6-bromoveratraldehyde (**14**) or with 6-chloropiperonal (**15**), which afforded the corresponding Schiff bases **16**–**19** in 82–95% yield. Those imines were subsequently reduced with sodium borohydride to the 5-quinolinamines and 5-isoquinolinamines **8**–**11** in almost quantitative yield. Alternatively, compounds **8** and **10** could be prepared in a one step procedure[27] by alkylation of 5-aminoquinoline (**12**) and 5-aminoisoquinoline (**13**) with 2-bromo-4,5-dimethoxybenzyl bromide (**20**).[28] Similarly, the use of 2-bromo-4,5-methylenedioxybenzyl bromide (**21**)[29] gave access to *N*-(2-bromo-4,5-methylenedioxybenzyl)-5-quinolinamine (**22**) and *N*-(2-bromo-4,5-methylenedioxybenzyl)-5-isoquinolinamine (**23**). This second approach gave lower overall yields than the first one, but was more versatile, since it also permitted the preparation of *N*-(2-bromo-5,6-dimethoxybenzyl)-5-isoquinolinamine (**24**) by condensation of 5-aminoisoquinoline (**13**) with 2-bromo-5,6-dimethoxybenzyl bromide (**25**).[30]

Cyclization of *N*-(2-halobenzyl)-5-quinolinamines **8** and **9** or **22**, and *N*-(2-halobenzyl)-5-isoquinolinamines **10**, **11** or **23**, and **24** using LDA in THF at −78 °C[25] was followed by spontaneous air-oxidation of the unstable dihydrophenanthroline intermediates during the work-up,[31] and gave the desired benzo[*c*][1,7]phenanthrolines **3** and **4**, and benzo[*c*][1,8]phenanthrolines **5**–**7**, respectively.

The study of the cytotoxic properties of the benzo[*c*]-phenanthrolines **3**–**7** was carried out in vitro on the L1210 murine leukemia cell line. The results (IC_{50}) are reported in Table 1. In both [1,7] and [1,8] series, the compounds bearing oxygenated substituents at positions 8 and 9 were found to be cytotoxic, with IC_{50} values within the same range of magnitude as fagaronine (**1**). In contrast, 7,8-dimethoxybenzo[*c*][1,8]phenanthroline (**7**) was about 10-fold less potent.

Table 1. Inhibition of L1210 cell proliferation and cell cycle perturbation induced by compounds **3**–**7** in comparison with fagaronine (**1**)

Compound	Cytotoxicity IC_{50} (μM)	Cell cycle perturbation[a]
1	6.4	35% G2 + M (25 μM)
3	7.1	44% G2 + M (25 μM)
4	8.8	61% G1 (50 μM)
5	6.6	76% G2 + M (25 μM)
6	4.4	61% G1 (10 μM)
7	43.1	Not tested

[a] Percentage of cells blocked in a specific phase of the cell cycle at the indicated concentration.

The perturbation of the cell cycle induced by compounds **3**–**6** was studied in the same cell line by flow cytometry. Interestingly, the cell cycle specificity observed depended on the nature of the oxygenated substituents on the benzo[*c*]phenanthroline basic core. Indeed, compounds **3** and **5**, both bearing two methoxy groups at positions 8 and 9, induced an accumulation in the G2 + M phases of the cell cycle, whereas compounds **4** and **6**, with a methylenedioxy substituent at the same positions induced an accumulation in the G1 phase, suggesting therefore the implications of at least two distinct mechanisms of action.

In an effort to elucidate their molecular mechanism of action, at least partially, compounds **3**–**6** were tested as potential inhibitors of topoisomerases I and II using conventional DNA relaxation assays.[32] No poisoning of human topoisomerase II was detected. Double stranded DNA cleavage, reflecting the stabilization of topoisomerase II–DNA covalent complexes, was observed with the reference drug etoposide but not with **3**–**6**, even when tested at concentrations up to 50 μM (data not shown). In contrast, drug-induced stabilization of topoisomerase I–DNA complexes was evidenced. The stimulation of topoisomerase I–mediated DNA single strand breaks was clearly shown with the reference drug camptothecin and to a lower but noticeable extent with **3**–**6** (Fig. 1a). The most efficient compound is the dimethoxy derivative **5**, which stimulated DNA cleavage by the enzyme in a concentration-dependent manner (Fig. 1b). The amount of nicked DNA species increased gradually with the increasing drug concentration but the poisoning effect remains weaker than that detected with camptothecin, by about 35% (Fig. 1c). In concentration-dependent experiments with camptothecin (data not shown), topoisomerase I inhibition starts to appear at about 1 μM whereas it requires a concentration 3–5 times higher to get a similar extent of DNA cleavage by topoisomerase I with compound **5**, which is the most potent of the four analogues tested. Moreover, even at high concentrations, the level of topoisomerase I inhibition seen with **5** never reaches that observed with camptothecin. The two dimethoxy derivatives **5** and **3** are both slightly more potent than the corresponding methylenedioxy analogs **4** and **6** at stabilizing topoisomerase I–DNA complexes.

DNA sequencing experiments were carried out to investigate further the inhibition of topoisomerase I by these compounds. In this case, the DNA substrate was a 117-bp restriction fragment labelled at the 3′-end with ^{32}P and subjected to cleavage by topoisomerase I in the absence or presence of the alkaloids at 10 or 50 μM. The cleavage products were resolved on polyacrylamide sequencing gels. A typical gel is presented in Figure 2. A few well resolved cleavage sites were identified with camptothecin, especially at the T·G positions 26, 48 and 81. These three main sites were also detected in the presence of the benzo[*c*]phenanthrolines **3**–**6** but cleavage intensities are considerably weaker. Here again, we found that **5** was the most efficient topoisomerase I poisons among the four compounds tested. The poisoning activity of this compound is clearly detected but

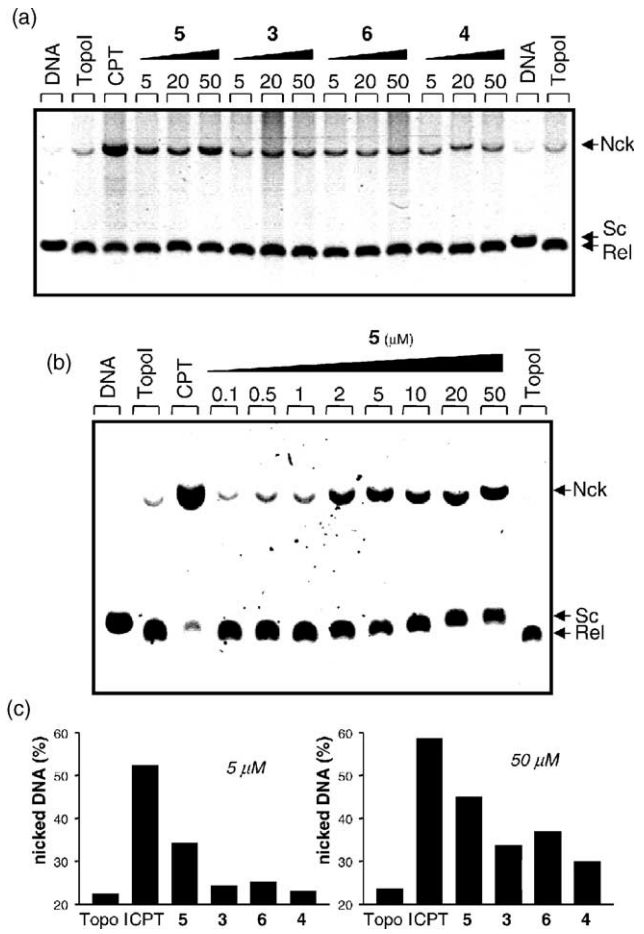


Figure 1. Topoisomerase I inhibition. (a) Effect of benzo[*c*]phenanthrolines **3**–**6** on the relaxation of plasmid DNA by topoisomerase I. Gel (b) shows the concentration-dependent effect of 5 tested at 0.1–50 μM, compared to CPT at 20 μM. Native supercoiled pLAZ3 DNA (0.3 μg) (lane DNA) was incubated with 4 units topoisomerase I in the absence (lane TopoI) or presence of drug at the indicated concentration (μM). Reactions were stopped with sodium dodecylsulfate and treatment with proteinase K. DNA samples were separated by electrophoresis on 1% agarose gels containing ethidium (1 μg/mL) and then photographed under UV light. Nck, nicked; Rel, relaxed; Sc, supercoiled DNA. (c) Comparison of the extent of topoisomerase I-mediated DNA cleavage measured with **3**–**6** or CPT. The histogram shows the formation of nicked DNA (form II, %) for each compound. Band intensities from three gels such as the one shown in panel (a) were compiled for the quantitative analysis.

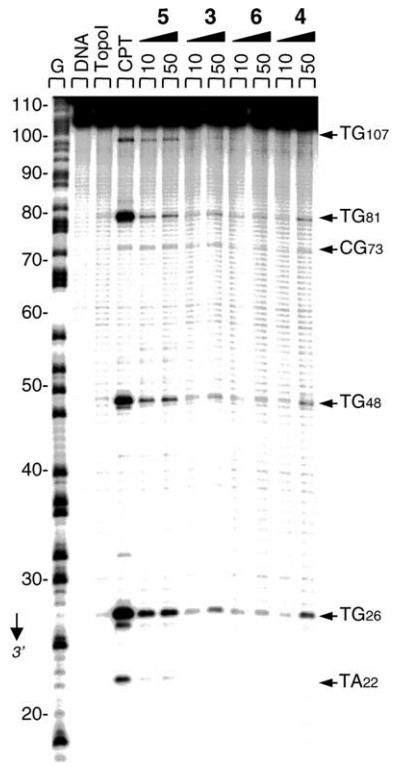


Figure 2. Cleavage of the 117-mer DNA fragment by topoisomerase I in the presence of the benzo[*c*]phenanthrolines **3**–**6**. The 3′-end labeled fragment (DNA) was incubated in the absence (lane TopoI) or presence of the alkaloids at 10 or 50 μM. Camptothecin (CPT) was used at 20 μM. Topoisomerase I cleavage reactions were analyzed on a 8% denaturing polyacrylamide gel. Numbers at the side of the gels show the nucleotide positions, determined with reference to the guanine track labeled G. The nucleotide positions and sequences of the main cleavage sites are indicated.

relatively discrete compared to the camptothecin. In other words, there is no doubt that this compound behaves as a conventional topoisomerase I poison in the molecular assays but its limited potency suggests that topoisomerase I is perhaps not its major target. This consideration prompted us to evaluate the cytotoxicity of **5** using a pair of murine leukemia cell lines, sensitive (P388) or resistant (P388CPT5) to camptothecin.[33,34] With this cell system, which we have previously employed with other toposiomerase I poisons,[35] we measured IC_{50} values of 3.1 and 2.4 μM for compound **5** on the P388 and P388CPT5 cell lines, respectively. The benzo[*c*][1,8]phenanthroline derivative is thus more or less equally toxic to the parental cells and the CPT-resistant cells expressing a mutated *top1* gene. For this reason, we believe that topoisomerase I cannot be considered as a primary target for the benzo[*c*]phenanthrolines tested here.

In parallel, we studied the interaction of the alkaloids with DNA by different spectroscopic methods. Binding to DNA was first visualized from absorption and fluorescence measurements (Fig. 3). Interaction of **5** with DNA causes bathochromic and hypochromic shifts and the titration shows a well resolved isosbestic point at 290 nm suggesting a homogeneous interaction (Fig. 3a). Similar titrations were observed by absorption spectroscopy with the other compounds although in these cases the spectral shifts were less pronounced (data not shown). The interaction with DNA was also evidenced by means of circular dichroism and fluorescence. A weak positive CD signal appeared at 320 nm upon interaction of **5** with DNA (spectra not shown) and in the fluorescence titration (excitation set at 300 nm), addition of DNA induces a pronounced quenching of the intrinsic fluorescence of **5** in the 350–450 nm region (Fig. 3b).

To determine the mode of binding of the compounds to DNA, we used electric linear dichroism (ELD), which is useful to probe the orientation of small molecules bound to nucleic acids.[36] A representative data set obtained

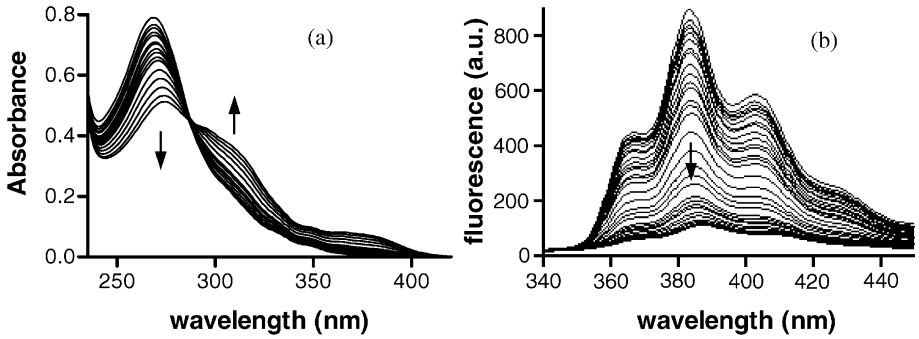


Figure 3. DNA titrations of **5** measured by (a) absorption and (b) fluorescence spectroscopy. To 1 mL of drug solution at (a) 20 μM or (b) 2.5 μM were added aliquots of a concentrated calf thymus DNA solution. The phosphate–DNA/drug ratio increased from 0 to 20 (top to bottom curves at (a) 270 nm or (b) 390 nm). For the fluorescence measurement, the excitation was set at 300 nm. Spectra were recorded in 1 mM sodium cacodylate buffer, pH 7.0.

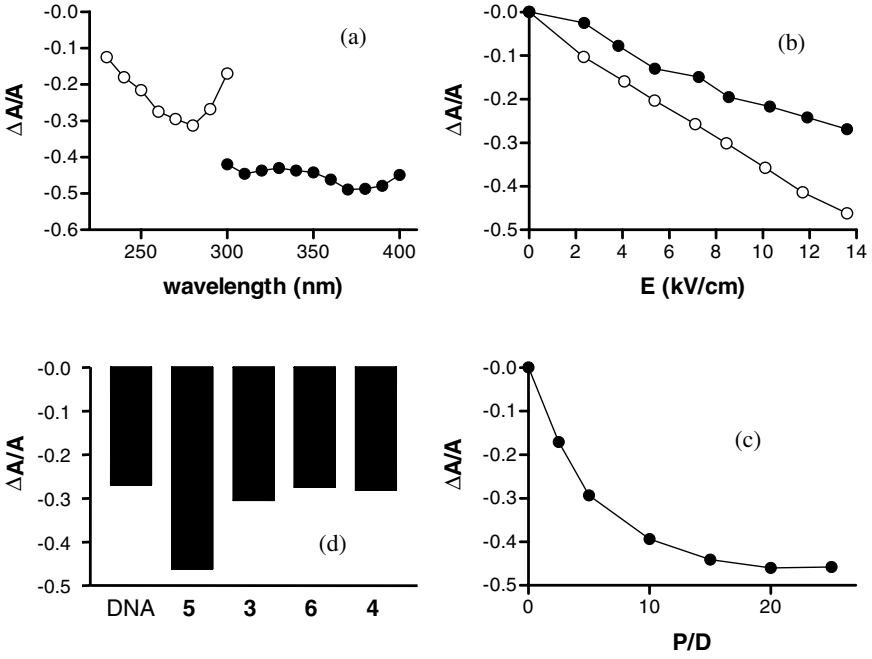


Figure 4. ELD data for the binding to DNA. Dependence of the reduced dichroism ΔA/A on (a) the wavelength, (b) the electric field strength and (c) the DNA–drug ratio (P/D) for (●) compound **5** bound to calf thymus DNA and (○) DNA alone. Conditions: (a) 13.6 kV/cm, P/D =20 (200 μM calf thymus DNA, 10 μM drug), (b) 360 nm, P/D =20 for the DNA–drug complexes and 260 nm for the DNA alone (c) 13.6 kV/cm, 360 nm, in 1 mM sodium cacodylate buffer, pH 7.0. The histograms in panel (d) compare the reduced dichroism values (ΔA/A) measured with DNA at 260 nm and the different drug–DNA complexes at 360 nm.

with **5** is presented in Figure 4a–c. The ELD spectrum of **5** bound to DNA exhibits negative reduced dichroism values (ΔA/A) in the 300–400 nm drug absorption band (where DNA does not absorb) (Fig. 4a) and the magnitude of the ΔA/A signal is dependent on both the electric field strength (Fig. 4b) and the DNA/drug ratio (Fig. 4c). The intensity of the ΔA/A signals measured at 360 nm with the different drug–DNA complexes is at least equal to that measured with DNA alone at 260 nm (Fig. 4d). Such strong negative signals are typical of DNA intercalating agents.[37] The more negative ΔA/A values recorded with **5** are often obtained with potent intercalating agents and likely reflect a drug-induced stiffening of the DNA macromolecule, which favours its orientation in the electric field. Altogether, the spectroscopic data are fully consistent with an intercalative binding mode, as expected for such planar molecules.

Intercalation of these benzo[*c*]phenanthrolines into DNA occurs independently of the target DNA sequence. DNase I footprinting experiments revealed no preferential binding sites (no footprints). These compounds interact equally well with AT and GC sequences. This is also observed by ELD. Binding of compound **5** to the polynucleotides poly(dAT)$_2$ and poly(dGC)$_2$ produced negative reduced dichroism signals at 360 nm of intensities comparable to those obtained with the polynucleotides alone at 260 nm (Fig. 5), indicating that

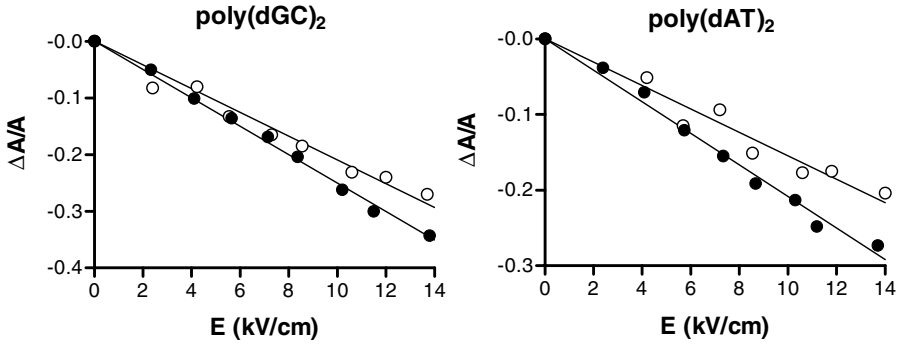


Figure 5. Variation of the reduced dichroism (ΔA/A) for compound **5** bound to poly(dAT)$_2$ or poly(dGC)$_2$, as a function of the electric field strength. ΔA/A was measured at 360 nm and at a DNA/drug ratio of 20 in 1 mM sodium cacodylate buffer, pH 7.0 symbols (●) and (○) refer to the drug–polynucleotide complex and the polynucleotide alone, respectively.

the compound can intercalate between both A–T and G–C base pairs.

3. Conclusion

We report an efficient strategy for the synthesis of novel benzo[*c*][1,7] and [1,8]phenanthrolines. In both series, compounds bearing oxygenated substituents at positions 8 and 9 exhibit interesting cytotoxic activities, which may result from their capacities to intercalate into DNA. Topoisomerase I inhibition was observed but apparently it does not constitute a primary event responsible for the cytotoxic action. Further mechanistic studies are needed to identify their molecular targets.

4. Experimental

4.1. General

Melting points were determined on a hot stage Reichert microscope and are uncorrected.Mass spectra were recorded with a Nermag R-10-10C spectrometer using desorption–chemical ionization (DCI–MS; reagent gas: NH_3). UV spectra (λ_{max} in nm) were recorded in spectroscopic grade MeOH on a Beckman Model 34 spectrophotometer. IR spectra (ν_{max} in cm^{-1}) were obtained from potassium bromide pellets or sodium chloride films on a Perkin–Elmer 257 instrument. ^1H NMR (δ [ppm], J [Hz]) spectra were run at 400 MHz and ^{13}C NMR spectra at 75 MHz, using Bruker AVANCE-400 and AC-300 spectrometers, respectively. When necessary, the structures of the novel compounds were insured and the signals unambiguously assigned by 2D NMR techniques: ^1H–^1H COSY, ^1H–^1H NOESY, ^{13}C–^1H HMQC, and ^{13}C–^1H HMBC. These experiments were performed using standard Bruker microprograms. Flash column chromatographies were conducted using silica gel 60 Merck (35–70 μM) with an overpressure of 300 mbars. Microanalyses were in agreement with calculated values ±0.4%. 5-Aminoquinoline (**12**) and 5-aminoisoquinoline (**13**) were purchased from Aldrich Chemical Company (Steinheim, Germany). 6-Bromoveratraldehyde (**14**) and 6-chloropiperonal (**15**) were purchased from Lancaster Synthesis Ltd (Morecambe, Lancashire, England).

4.2. General procedure for the synthesis of imines 16–19

An solution of 5-aminoquinoline (**12**) or 5-aminoisoquinoline (**13**) (101 mg, 0.7 mmol) and 6-bromoveratraldehyde (**14**) (172 mg, 0.7 mmol) or 6-chloropiperonal (**15**) (129 mg, 0.7 mmol) in 1,2-dichlorobenzene (30 mL) was refluxed in a Dean–Stark apparatus for 24 h. The solvent was evaporated under reduced pressure. Crystallization of the residue from toluene afforded the imines **16**–**19** as pale yellow prisms.

4.3. *N*-(Quinolin-5-yl)-2-bromo-4,5-dimethoxyphenylmethanimine (16)

Yield 82%, mp 207 °C (from toluene), UV λ (MeOH) 238, 285 nm; IR (KBr) ν 2910, 1595, 1514, 1440, 1262, 1024 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.90 (6H, s, 2 OCH_3), 7.10 (1H, s, H-3′), 7.15 (1H, dd, J = 7, 2, H-6), 7.42 (1H, dd, J = 8.5, 4, H-3), 7.57 (1H, dd, J = 8, 2, H-8), 7.71 (1H, dd, J = 8, 7, H-7), 7.90 (1H, s, H-6′), 8.66 (1H, dd, J = 8.5, 2, H-4), 8.88 (1H, s, H-7′), 8.92 (1H, dd, J = 4, 2, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0 (OCH_3), 56.2 (OCH_3), 110.2 (C-6), 113.5 (C-6′), 115.2 (C-3′), 118.5 (C-2′), 124.2 (C-3), 127.1 (C-8), 128.7 (C-4a), 129.6 (C-4), 130.8 (C-1′), 132.5 (C-7), 148.5 (C-8a), 148.9 (2C, C-4′, C-5′), 150.8 (C-2), 152.6 (C-5), 159.8 (C-7′); DCI–MS m/z 371, 373 $[MH]^+$. Anal. ($C_{18}H_{15}BrN_2O_2$): C, H, Br, N.

4.4. *N*-(Quinolin-5-yl)-2-chloro-4,5-methylenedioxyphenylmethanimine (17)

Yield 85%, mp 266 °C (from toluene), UV λ (MeOH) 287 nm; IR (KBr) ν 2923, 1585, 1570, 1504, 1482, 1254, 1120, 1050, 922, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$/CD_3OD 9:1) δ 6.25 (2H, s, OCH_2O), 7.30 (1H, s, H-3′), 7.40 (1H, dd, J = 7, 1, H-6), 7.60 (1H, dd, J = 8.5, 4, H-3), 7.80 (1H, dd, J = 8, 7, H-7), 7.90 (1H, s, H-6′), 7.95 (1H, dd, J = 8, 1, H-8), 8.77 (1H, dd, J = 8.5, 2, H-4), 8.95 (1H, s, H-7′), 9.00 (1H, dd, J = 4, 2, H-2); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD 9:1) δ 102.3 (OCH_2O),

106.9 (C-6), 109.8 (C-6′), 113.5 (C-3′), 120.7 (C-3), 124.3 (C-2′), 124.5 (C-3), 126.8 (C-8), 126.9 (C-4a), 129.9 (C-4), 130.5 (C-1′), 133.2 (C-7), 147.3 (C-8a), 147.7 (C-5′), 148.7 (C-4′), 150.2 (C-2), 151.2 (C-5), 158.2 (C-7′); DCI–MS m/z 311, 313 $[MH]^+$. Anal. ($C_{17}H_{11}ClN_2O_2$): C, H, Cl, N.

4.5. *N*-(Isoquinolin-5-yl)-2-bromo-4,5-dimethoxyphenylmethanimine (18)

Yield 90%, mp 168–169 °C (from toluene), UV λ (MeOH) 285, 340 nm; IR (NaCl) ν 2923, 1482, 1254, 1120, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.85 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 7.09 (1H, s, H-3′), 7.30 (1H, dd, $J = 7$, 1, H-6), 7.62 (1H, dd, $J = 8$, 7, H-7), 7.87 (1H, dd, $J = 8$, 1, H-8), 7.92 (1H, s, H-6′), 8.12 (1H, d, $J = 6$, H-4), 8.58 (1H, d, $J = 6$, H-3), 8.83 (1H, s, H-7′), 9.28 (1H, s, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1 (OCH_3), 56.3 (OCH_3), 108.3 (C-6′), 113.1 (C-3′), 114.7 (C-6), 115.0 (C-4), 116.5 (C-2′), 123.1 (C-8), 124.9 (C-1′), 125.5 (C-7), 126.9 (C-8a), 129.6 (C-4a), 141.0 (C-3), 146.0 (C-5′), 146.8 (C-4′), 150.1 (C-1), 150.6 (C-5), 157.9 (C-7′); DCI–MS m/z 371, 373 $[MH]^+$. Anal. ($C_{18}H_{15}BrN_2O_2$): C, H, Br, N.

4.6. *N*-(Isoquinolin-5-yl)-2-chloro-4,5-methylenedioxyphenylmethanimine (19)

Yield 95%, mp 167 °C (from toluene), UV λ (MeOH) 244, 274, 340 nm; IR (NaCl) ν 2910, 1585, 1477, 1383, 1248, 1040 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.93 (2H, s, OCH_2O), 7.09 (1H, s, H-3′), 7.27 (1H, dd, $J = 7$, 1, H-6), 7.61 (1H, dd, $J = 8$, 7, H-7), 7.86 (1H, dd, $J = 8$, 1, H-8), 7.88 (1H, s, H-6′), 8.11 (1H, d, $J = 6$, H-4), 8.57 (1H, d, $J = 6$, H-3), 8.91 (1H, s, H-7′), 9.27 (1H, s, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 101.8 (OCH_2O), 108.6 (C-6′), 110.3 (C-3′), 113.4 (C-2′), 116.9 (2C, C-4, C-6), 125.2 (C-1′), 126.2 (C-8a), 128.2 (C-8), 128.9 (C-7), 129.4 (C-4a), 141.9 (C-3), 142.3 (2C, C-4′, C-5′), 147.2 (C-1), 147.6 (C-5), 153.1 (C-7′); DCI–MS m/z 311, 313 $[MH]^+$. Anal. ($C_{17}H_{11}ClN_2O_2$): C, H, Cl, N.

4.7. General procedure for the reduction of imines 16–19

Sodium borohydride (1 g, 26 mmol) was added over 30 min to a solution of the imine (1.6 mmol) in MeOH (100 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h. The solvent was evaporated under reduced pressure. Water (150 mL) and CH_2Cl_2 (150 mL) were added to the residue. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. Crystallization from toluene gave the *N*-benzylquinolinamines **8** and **9**, and the *N*-benzylisoquinolinamines **10** and **11** as pale yellow crystals.

4.8. *N*-(2-Bromo-4,5-dimethoxybenzyl)-5-quinolinamine (8)

Yield 96%, mp 214 °C (from toluene), UV λ (MeOH) 256, 288, 328 nm; IR (KBr) ν 3400, 2964, 2836, 1588, 1474, 901 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.75 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.52 (2H, d, $J = 5$, CH_2-7′), 4.70 (1H, t, $J = 5$, D_2O exch., NH), 6.66 (1H, dd, $J = 7$, 2, H-6), 6.95 (1H, s, H-6′), 7.08 (1H, s, H-3′), 7.37 (1H, dd, $J = 8.5$, 4, H-3), 7.57 (2H, m, H-7, H-8), 8.25 (1H, dd, $J = 8.5$, 1, H-4), 8.90 (1H, dd, $J = 4$, 1, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 48.6 (C-7′), 56.0 (OCH_3), 56.2 (OCH_3), 105.5 (C-6), 112.4 (C-6′), 113.5 (C-2′), 115.7 (C-3′), 118.5 (C-4a), 119.0 (C-3), 119.4 (C-8), 128.7 (C-4), 129.3 (C-1′), 130.3 (C-7), 143.1 (C-5), 148.6 (C-8a), 148.9 (C-5′), 149.1 (C-4′), 149.9 (C-2); DCI–MS m/z 373, 375 $[MH]^+$. Anal. ($C_{18}H_{17}BrN_2O_2$): C, H, Br, N.

4.9. *N*-(2-Chloro-4,5-methylenedioxybenzyl)-5-quinolinamine (9)

Yield 97%, mp 259 °C (from toluene), UV λ (MeOH) 237, 346 nm; IR (NaCl) ν 3400, 2955, 2925, 1597, 1486, 1266, 1052, 797 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$/CD_3OD 9:1) δ 4.51 (2H, d, $J = 5$, CH_2-7′), 4.80 (1H, t, $J = 5$, D_2O exch., NH), 5.98 (2H, s, OCH_2O), 6.60 (1H, dd, $J = 6$, 2, H-6), 6.91 (1H, s, H-3′, H-6′), 7.35 (1H, dd, $J = 8$, 4, H-3), 7.58 (2H, m, H-7, H-8), 8.22 (1H, dd, $J = 8$, 2, H-4), 8.89 (1H, dd, $J = 4$, 2, H-2); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD 9:1) δ 48.7 (C-7′), 101.5 (OCH_2O), 105.5 (C-6), 113.2 (C-6′), 113.4 (C-3′), 118.5 (C-4a), 120.6 (C-3), 124.3 (C-2′), 127.8 (C-8), 129.9 (C-4), 130.8 (C-1′), 133.2 (C-7), 142.8 (C-5), 147.5 (C-8a), 147.7 (C-5′), 147.8 (C-4′), 150.1 (C-2); DCI–MS m/z 313, 315 $[MH]^+$. Anal. ($C_{17}H_{13}ClN_2O_2$): C, H, Cl, N.

4.10. *N*-(2-Bromo-4,5-dimethoxybenzyl)-5-isoquinolinamine (10)

Yield 92%, mp 245 °C (from toluene), UV λ (MeOH) 245, 285, 345 nm; IR (KBr) ν 3410, 2925, 2853, 1583, 1503, 1385, 1263, 1030 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.75 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.50 (2H, d, $J = 5$, CH_2-7′), 4.85 (1H, t, $J = 5$, D_2O exch., NH), 6.74 (1H, d, $J = 7$, H-6), 6.95 (1H, s, H-6′), 7.07 (1H, s, H-3′), 7.35 (1H, d, $J = 8$, H-8), 7.45 (1H, dd, $J = 8$, 7, H-7), 7.61 (1H, d, $J = 6$, H-4), 8.48 (1H, d, $J = 6$, H-3), 9.15 (1H, s, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 48.5 (C-7′), 56.1 (OCH_3), 56.3 (OCH_3), 108.3 (C-6), 113.2 (C-6′), 114.7 (C-4), 115.0 (C-3′), 116.5 (C-2′), 125.5 (C-8), 126.1 (C-4a), 128.0 (C-7), 129.3 (2C, C-1′, C-8a), 142.0 (C-5), 142.1 (C-3), 148.7 (2C, C-4′, C-5′), 153.0 (C-1); DCI–MS m/z 373, 375 $[MH]^+$. Anal. ($C_{18}H_{17}BrN_2O_2$): C, H, Br, N.

4.11. *N*-(2-Chloro-4,5-methylenedioxybenzyl)-5-isoquinolinamine (11)

Yield 90%, mp 161 °C (from toluene), UV λ (MeOH) 239, 300, 333 nm; IR (NaCl) ν 3326, 2955, 1586, 1476, 1368, 1248, 1039, 789 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.50 (2H, d, $J = 5$, CH_2-7′), 4.88 (1H, t, $J = 5$, D_2O exch., NH), 5.95 (2H, s, OCH_2O), 6.71 (1H, d, $J = 7$, H-6), 6.89 (1H, s, H-6′), 6.90 (1H, s, H-3′), 7.32 (1H, d, $J = 8$, H-8), 7.45 (1H, dd, $J = 8$, 7, H-7), 7.62

(1H, d, $J = 6$, H-4), 8.53 (1H, d, $J = 6$, H-3), 9.25 (1H, s, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 45.8 (C-7′), 101.8 (OCH_2O), 108.2 (C-6), 108.4 (C-6′), 108.7 (C-4), 111.2 (C-3′), 116.7 (C-8), 125.0 (C-2′), 125.9 (C-4a), 128.0 (C-7), 128.8 (C-1′), 129.3 (C-8a), 141.8 (C-5), 142.1 (C-3), 147.1 (C-5′), 147.5 (C-4′), 152.9 (C-1); DCI–MS m/z 313, 315 $[MH]^+$. Anal. ($C_{17}H_{13}ClN_2O_2$): C, H, Cl, N.

4.12. General procedure for the one step synthesis of *N*-benzylquinolinamines 8 and 22, and *N*-benzylisoquinolinamines 10, 23 and 24

A mixture of 5-aminoquinoline (**12**) or 5-aminoisoquinoline (**13**) (0.69 g, 4.85 mmol) and neutral alumina (9.65 g) in anhydrous toluene (140 mL) was stirred for 30 min at room temperature under argon. The respective 2-bromo-dialkoxybenzyl bromide (2-bromo-4,5-dimethoxybenzyl bromide (**20**),[27] 2-bromo-4,5-methylenedioxybenzyl bromide (**21**),[28] or 2-bromo-5,6-dimethoxybenzyl bromide (**25**)[29]) (4.85 mmol) was added, and the suspension was refluxed under argon for 48 h. After addition of water (50 mL), the mixture was stirred for 30 min and the alumina was filtered off. The filtrate was extracted with CH_2Cl_2 (3×75 mL). The organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography of the residue over silica gel (eluent: CH_2Cl_2/MeOH 99:1) gave *N*-benzylquinolinamines **8** and **22**, and *N*-benzylisoquinolinamines **10**, **23** and **24** in 15–35% yield.

4.13. *N*-(2-Bromo-4,5-methylenedioxybenzyl)-5-quinolinamine (22)

Yield 21%, mp 209 °C (from toluene), UV λ (MeOH) 229, 330 nm; IR (KBr) ν 3407, 2952, 1576, 1443, 1273, 1074, 798 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.51 (2H, d, $J = 5$, CH_2-7′), 4.80 (1H, t, $J = 5$, D_2O exch., NH), 5.93 (2H, s, OCH_2O), 6.60 (1H, dd, $J = 7$, 2, H-6), 6.90 (1H, s, H-6′), 7.05 (1H, s, H-3′), 7.32 (1H, dd, $J = 8.5$, 4, H-3), 7.57 (2H, m, H-7, H-8), 8.22 (1H, dd, $J = 8.5$, 1, H-4), 8.90 (1H, dd, $J = 4$, 1, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 48.7 (C-7′), 101.8 (OCH_2O), 105.5 (C-6), 113.0 (C-6′), 113.7 (C-3′), 114.3 (C-2′), 118.5 (C-4a), 119.2 (C-3), 119.5 (C-8), 128.6 (C-4), 130.3 (C-7), 130.7 (C-1′), 142.8 (C-5), 147.7 (C-5′), 147.8 (C-4′), 149.3 (C-8a), 150.1 (C-2); DCI–MS m/z 357, 359 $[MH]^+$. Anal. ($C_{17}H_{13}BrN_2O_2$): C, H, Br, N.

4.14. *N*-(2-Bromo-4,5-methylenedioxybenzyl)-5-isoquinolinamine (23)

Yield 17%, mp 202 °C (from toluene), UV λ (MeOH) 222, 274, 333 nm; IR (KBr) ν 3326, 1585, 1103, 931 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.47 (2H, d, $J = 5$, CH_2-7′), 5.00 (1H, t, $J = 5$, D_2O exch., NH), 5.93 (2H, s, OCH_2O), 6.68 (1H, d, $J = 7$, H-6), 6.89 (1H, s, H-6′), 7.05 (1H, s, H-3′), 7.32 (1H, d, $J = 8$, H-8), 7.42 (1H, dd, $J = 8$, 7, H-7), 7.62 (1H, d, $J = 6$, H-4), 8.55 (1H, d, $J = 6$, H-3), 9.25 (1H, s, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 45.9 (C-7′), 101.8 (OCH_2O), 108.4 (C-6), 108.3 (C-2′), 108.8 (C-6′), 110.9 (C-4), 111.3 (C-3′), 113.4 (C-4a), 116.8 (C-8), 127.8 (C-8a), 128.1 (C-7), 128.8 (C-1′), 141.7 (C-5), 142.0 (C-3), 145.7 (2C, C-4′, C-5′), 152.9 (C-1); DCI–MS m/z 357, 359 $[MH]^+$. Anal. ($C_{17}H_{13}BrN_2O_2$): C, H, Br, N.

4.15. *N*-(2-Bromo-5,6-dimethoxybenzyl)-5-isoquinolinamine (24)

Yield 34%, amorphous solid, UV λ (MeOH) 330 nm; IR (KBr) ν 3254, 3065, 2883, 1575, 1335, 789 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.82 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.61 (2H, d, $J = 5$, CH_2-7′), 4.85 (1H, t, $J = 5$, D_2O exch., NH), 6.81 (1H, d, $J = 9$, H-4′), 7.10 (1H, d, $J = 8$, H-6), 7.30 (1H, d, $J = 9$, H-3′), 7.35 (1H, d, $J = 8$, H-8), 7.49 (1H, t, $J = 8$, H-7), 7.57 (1H, d, $J = 6$, H-4), 8.45 (1H, d, $J = 6$, H-3), 9.17 (1H, s, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 42.9 (C-7′), 55.8 (OCH_3), 61.6 (OCH_3), 108.4 (C-6), 113.1 (C-4), 113.4 (C-4′), 115.3 (C-2′), 116.3 (C-8), 126.2 (C-4a), 128.0 (C-3′), 128.1 (C-7), 129.3 (C-8a), 131.7 (C-1′), 141.9 (C-3), 142.2 (C-5), 148.8 (C-5′), 152.3 (C-6′), 152.7 (C-1); DCI–MS m/z 373, 375 $[MH]^+$. Anal. ($C_{18}H_{17}BrN_2O_2$): C, H, Br, N.

4.16. General procedure for the synthesis of benzo[*c*]-phenanthroline derivatives 3–7

A 2 M lithium diisopropylamide solution in THF (2 mL, 4.02 mmol) was added at −78 °C under argon to a solution of the respective *N*-benzylquinolinamine or *N*-benzylisoquinolinamine (0.67 mmol) in anhydrous THF (16 mL). The reaction mixture was stirred at −78 °C for 4 h and allowed to warm to room temperature within 16 h. The solution was evaporated to dryness in vacuo and the residue was dissolved in CH_2Cl_2 (50 mL) and washed with H_2O (50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography of the residue over silica gel (eluent: CH_2Cl_2/MeOH 90:10) afforded the benzo[*c*]phenanthrolines **3**–**7**.

4.17. 8,9-Dimethoxybenzo[*c*][1,7]phenanthroline (3)

Yield 63%, amorphous solid, UV λ nm (MeOH) ($\log \varepsilon$) 212 (4.09), 275.5 (4.50), 360 (3.13); IR (KBr) ν 2970, 2960, 1521, 1496, 1397, 12861170, 1152, 819 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (3H, s, OCH_3), 4.20 (3H, s, OCH_3), 7.39 (1H, s, H-7), 7.62 (1H, dd, $J = 8$, 4, H-3), 7.89 (1H, s, H-10), 8.22 (1H, d, $J = 9$, H-12), 8.63 (1H, d, $J = 9$, H-11), 9.04 (1H, dd, $J = 4$, 2, H-2), 9.28 (1H, s, H-6), 9.60 (1H, dd, $J = 8$, 2, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.2 (2C, 2 OCH_3), 101.7 (C-10), 107.2 (C-7), 120.7 (C-10b), 121.8 (C-3), 122.9 (C-6a), 123.5 (C-11), 127.5 (C-4a), 128.4 (C-12), 128.5 (C-10a), 132.9 (C-4), 140.3 (C-4b), 148.4 (C-12a), 150.3 (C-8), 150.6 (2C, C-2, C-6), 153.3 (C-9); DCI–MS m/z 291 $[MH]^+$. Anal. ($C_{18}H_{14}N_2O_2$): C, H, N.

4.18. 8,9-Methylenedioxybenzo[*c*][1,7]phenanthroline (4)

Yield 51%, amorphous solid, UV λ nm (MeOH) (log ε) 211 (3.15), 275 (3.58), 343 (2.40), 360 (2.29); IR (KBr) ν 2950, 1471, 1446, 1262, 1034, 829 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.30 (2H, s, OCH_2O), 7.33 (1H, s, H-7), 7.62 (1H, dd, $J = 8$, 4, H-3), 8.15 (1H, d, $J = 9$, H-12), 8.43 (1H, s, H-10), 8.95 (1H, d, $J = 9$, H-11), 9.03 (1H, dd, $J = 4$, 1, H-2), 9.40 (1H, s, H-6), 9.54 (1H, dd, $J = 8$, 1, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 101.5 (C-10), 102.0 (OCH_2O), 105.1 (C-7), 121.3 (C-10b), 121.8 (C-3), 123.6 (C-6a), 123.7 (C-11), 127.5 (C-4a), 128.6 (C-12), 130.6 (C-10a), 133.0 (C-4), 140.6 (C-4b), 148.5 (C-12a), 150.7 (2C, C-2, C-6), 152.1 (2C, C-8, C-9); DCI–MS m/z 275 $[MH]^+$. Anal. ($C_{17}H_{10}N_2O_2$): C, H, N.

4.19. 8,9-Dimethoxybenzo[*c*][1,8]phenanthroline (5)

Yield 56%, amorphous solid, UV λ nm (MeOH) (log ε) 258 (2.75), 341 (2.45); IR (KBr) ν 2963, 2925, 1616, 1520, 1488, 1422, 1277, 1030, 850, 802 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.09 (3H, s, OCH_3), 4.13 (3H, s, OCH_3), 7.38 (1H, s, H-7), 7.82 (1H, s, H-10), 7.98 (1H, d, $J = 9$, H-12), 8.42 (1H, d, $J = 9$, H-11), 8.81 (1H, d, $J = 6$, H-3), 8.96 (1H, d, $J = 6$, H-4), 9.27 (1H, s, H-6), 9.53 (1H, s, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.2 (2C, 2 OCH_3), 101.6 (C-10), 107.1 (C-7), 117.4 (C-4), 121.3 (C-11), 123.2 (C-10b), 123.4 (C-6a), 125.3 (C-12), 127.6 (C-12a), 128.1 (C-10a), 136.0 (C-4a), 139.0 (C-4b), 144.8 (C-3), 150.5 (C-6), 150.9 (C-1), 153.2 (2C, C-8, C-9); DCI–MS m/z 291 $[MH]^+$. Anal. ($C_{18}H_{14}N_2O_2$): C, H, N.

4.20. 8,9-Methylenedioxybenzo[*c*][1,8]phenanthroline (6)

Yield 35%, amorphous solid, UV λ nm (MeOH) (log ε) 218 (2.98), 279 (3.15), 306 (2.83); IR (KBr) ν 2918, 1628, 1525, 1460, 1255, 1029, 855 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.30 (2H, s, OCH_2O), 7.76 (1H, s, H-7), 8.21 (1H, d, $J = 9$, H-12), 8.44 (1H, s, H-10), 8.76 (1H, d, $J = 6$, H-3), 8.83 (1H, d, $J = 9$, H-11), 8.95 (1H, d, $J = 6$, H-4), 9.40 (1H, s, H-6), 9.42 (1H, s, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 101.7 (C-10), 103.6 (OCH_2O), 106.1 (C-7), 117.9 (C-4), 123.9 (C-11), 124.9 (2C, C-6a, C-10b), 125.8 (C-12a), 126.6 (C-12), 128.8 (C-10a), 136.0 (C-4a), 139.6 (C-4b), 146.0 (C-3), 150.0 (C-8), 152.3 (C-6), 152.5 (C-1), 153.0 (C-9); DCI–MS m/z 275 $[MH]^+$. Anal. ($C_{17}H_{10}N_2O_2$): C, H, N.

4.21. 7,8-Dimethoxybenzo[*c*][1,8]phenanthroline (7)

Yield 28%, amorphous solid, UV λ nm (MeOH) (log ε) 270 (3.46), 307 (2.83); IR (KBr) ν 2964, 2930, 1577, 1459, 1279, 1085, 1017, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$)δ 4.10 (3H, s, OCH_3), 4.12 (3H, s, OCH_3), 7.76 (1H, d, $J = 9$, H-9), 8.06 (1H, d, $J = 9$, H-12), 8.44 (1H, d, $J = 9$, H-10), 8.56 (1H, d, $J = 9$, H-11), 8.83 (1H, d, $J = 6$, H-3), 9.05 (1H, d, $J = 6$, H-4), 9.28 (1H, s, H-1), 9.82 (1H, s, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.7 (OCH_3), 61.9 (OCH_3), 117.3 (C-4), 118.7 (C-10), 118.8 (C-9), 121.4 (C-11), 122.9 (C-6a), 123.6 (C-10b), 126.1 (C-12), 127.2 (C-12a), 128.7 (C-10a), 135.9 (C-4a), 138.7 (C-4b), 145.1 (C-3), 147.7 (C-6), 150.4 (2C, C-7, C-8), 151.3 (C-1); DCI–MS m/z 291 $[MH]^+$. Anal. ($C_{18}H_{14}N_2O_2$): C, H, N.

4.22. Cytotoxicity

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 μg/mL streptomycin and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured by microculture tetrazolium assay essentially as described.[38] Cells were exposed for 48 h to nine graded concentrations in triplicate of the test drug. Results are expressed as IC_{50} (mean, $n = 3$), which is defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells.

4.23. Cell cycle analysis

For the cell cycle analysis, L1210 cells (5×105 cells/mL) were incubated for 21 h with various concentrations of drugs. Cells were then fixed by 70% ethanol (v/v), washed, and incubated in PBS containing 100 μg/mL RNAse and 50 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10,000 cells were analyzed on an XLMCL flow cytometer (Beckman Coulter, France). Results are expressed as % of cells arrested in the given phases of the cell cycle.

4.24. Topoisomerase I inhibition

Recombinant topoisomerase I protein was produced and purified from baculovirus infected Sf9 cells.[39] Supercoiled pKMp27 DNA (0.4 μg) was incubated with 4 units topoisomerase I at 37 °C for 1 h in relaxation buffer (50 mM Tris pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA) in the presence of varying concentrations of the drug under study. Reactions were terminated by adding SDS to 0.25% and proteinase K to 250 μg/mL. DNA samples were then added to the electrophoresis dye mixture (3 μL) and electrophoresed in an ethidium-containing 1% agarose gel at room temperature for 2 h at 120 V. Gels were, washed and photographed under UV light.[32]

4.25. Purification of the DNA restriction fragment and radiolabeling

The 117-bp DNA fragment was prepared by 3′-[^{32}P]-end labeling of the *Eco* RI-*Pvu* II double digest of the pBS plasmid (Stratagene) using α-[^{32}P]-dATP (Amersham, 3000 Ci/mmol) and AMV reverse transcriptase (Roche). The labeled digestion products were separated on a 6% polyacrylamide gel under nondenaturing conditions in TBE buffer (89 mM Tris–borate pH 8.3, 1 mM EDTA).

After autoradiography, the requisite band of DNA was excised, crushed and soaked in water overnight at 37 °C. This suspension was filtered through a Millipore 0.22 μM filter and the DNA was precipitated with ethanol. Following washing with 70% ethanol and vacuum drying of the precipitate, the labeled DNA was re-suspended in 10 mM Tris adjusted to pH 7.0 containing 10 mM NaCl.

4.26. Sequencing of topoisomerase I-mediated DNA cleavage sites

Each reaction mixture contained 2 μL of 3′-end [^{32}P] labeled DNA (~1 μM), 5 μL of water, 2 μL of 10X topoisomerase I buffer, 10 μL of drug solution at the desired concentration (50 μM final concentration). After 10 min incubation to ensure equilibration, the reaction was initiated by addition of 2 μL (20 units) topoisomerase I. Samples were incubated for 45 min at 37 °C prior to adding SDS to 0.25% and proteinase K to 250 μg/mL to dissociate the drug–DNA–topoisomerase I cleavable complexes. The DNA was precipitated with ethanol and then resuspended in 5 μL of formamide–TBE loading buffer, denatured at 90 °C for 4 min then chilled in ice for 4 min prior to loading on to the sequencing gel. DNA cleavage products were resolved by polyacrylamide gel electrophoresis under denaturing conditions (0.3 mm thick, 8% acrylamide containing 8 M urea). After electrophoresis (about 2.5 h at 60 Watts, 1600 V in TBE buffer), gels were soaked in 10% acetic acid for 10 min, transferred to Whatman 3 MM paper, and dried under vacuum at 80 °C. A Molecular Dynamics 425E PhosphorImager was used to collect data from the storage screens exposed to dried gels overnight at room temperature. Each resolved band was assigned to a particular bond within the DNA fragment by comparison of its position relative to sequencing standards generated by treatment of the DNA with dimethylsulfate followed by piperidine-induced cleavage at the modified guanine residues.

4.27. Absorption spectroscopy

Absorption spectra were recorded with an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. Titrations of the drug with DNA, covering a large range of DNA–phosphate/drug ratios (P/D), were performed by adding aliquots of a concentrated DNA solution to a drug solution at constant ligand concentration (20 μM)in 1 mM sodium cacodylate buffer, pH 7.0.

4.28. Fluorescence titration experiments

Fluorescence titration data were recorded at room temperature using a Perkin–Elmer LS50B fluorometer. Excitation was at 300 nm and fluorescence emission was monitored over the range 320–570 nm. Samples used for titration experiments were prepared separately at a constant drug concentration (2.5 μM), and DNA concentrations ranging from 0.1–500 μM bp in 1 mM sodium cacodylate buffer, pH 7.0.

4.29. Electric linear dichroism

Calf thymus DNA (Pharmacia) was deproteinized with sodium dodecyl sulfate (protein content <0.2%) and extensively dialyzed against 1 mM sodium cacodylate buffered solution pH 7.0. ELD measurements were performed with a computerized optical measurement system using the procedures previously outlined.[40] All experiments were conducted with a 10 mm pathlength Kerr cell having 1.5 mm electrode separation. The samples were oriented under an electric field strength varying from 1 to 14 kV/cm. The drug under test was present at 10 μM concentration together with the DNA at 200 μM concentration unless otherwise stated. This electro-optical method has proved most useful to determine the orientation of the drugs bound to DNA. It has the additional advantage that it senses only the orientation of the polymer-bound ligand: free ligand is isotropic and does not contribute to the signal.[36]

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References and notes

1. Wang, J. C. *Annu. Rev. Biochem.* **1985**, *54*, 665.
2. Chen, A. Y.; Liu, L. F. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 191.
3. Liu, F. L. *Annu. Rev. Biochem.* **1989**, *58*, 351.
4. Li, T.-K.; Liu, F. L. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 53.
5. Bailly, C. *Curr. Med. Chem.* **2000**, *7*, 39.
6. Lerchen, H.-G. *Drugs Fut.* **2002**, *27*, 869.
7. Arcamone, F.; Animati, F.; Capranico, G.; Lombardi, P.; Pratesi, G.; Manzini, S.; Supino, R.; Zunino, F. *Pharmacol. Ther.* **1997**, *76*, 117.
8. Gewirtz, D. A. *Biochem. Pharmacol.* **1999**, *57*, 727.
9. Messmer, W. M.; Tin-Wa, M.; Fong, H. H. S.; Bevelle, C.; Farnsworth, N. R.; Abraham, D. J.; Trojánek, J. *J. Pharm. Sci.* **1972**, *61*, 1858.
10. Barret, Y.; Sauvaire, Y. *Phytother. Res.* **1992**, *6*, 59.
11. Fang, S.-D.; Wang, L.-K.; Hecht, S. M. *J. Org. Chem.* **1993**, *58*, 5025.
12. Kragh Larsen, A.; Grondard, L.; Couprie, J.; Desoize, B.; Comoe, L.; Jardillier, J.-C.; Riou, J.-F. *Biochem. Pharmacol.* **1993**, *46*, 1403.
13. Wang, L.-K.; Johnson, R. K.; Hecht, S. M. *Chem. Res. Toxicol.* **1993**, *6*, 813.
14. Holden, J. A.; Wall, M. E.; Wani, M. C.; Manikumar, G. *Arch. Biochem. Biophys.* **1999**, *370*, 66.

15. Douros, J.; Suffness, M. *Recent Results Cancer Res.* **1981**, *76*, 153.
16. Janin, Y. L.; Croisy, A.; Riou, J.-F.; Bisagni, E. *J. Med. Chem.* **1993**, *36*, 3686.
17. Li, D.; Zhao, B.; Sim, S. P.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 521.
18. Makhey, D.; Li, D.; Zhao, B.; Sim, S. P.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 1809.
19. Li, D.; Zhao, B.; Sim, S.-P.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 3795.
20. Strumberg, D.; Pommier, Y.; Paull, K.; Jayarama, M.; Nagafuji, P.; Cushman, M. *J. Med. Chem.* **1999**, *42*, 446.
21. Ruchelman, A. L.; Singh, S. K.; Ray, A.; Wu, X. H.; Yang, J.-M.; Li, T.-K.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 2061.
22. Singh, S. K.; Ruchelman, A. L.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *J. Med. Chem.* **2003**, *46*, 2254.
23. Poddevin, B.; Riou, J. F.; Lavelle, F.; Pommier, Y. *Mol. Pharmacol.* **1993**, *44*, 767.
24. Riou, J. F.; Fosse, P.; Nguyen, C. H.; Larsen, A. K.; Bissery, M. C.; Grondard, L.; Saucier, J. M.; Bisagni, E.; Lavelle, F. *Cancer Res.* **1993**, *53*, 5987.
25. Kessar, S. V.; Gupta, Y. P.; Balakrishnan, P.; Sawal, K. K.; Taj, M.; Dutt, M. *J. Org. Chem.* **1988**, *53*, 1708.
26. Razafimbelo, J.; Baudouin, G.; Tillequin, F.; Renard, P.; Léonce, S.; Pierré, A.; Atassi, Gh. *Chem. Pharm. Bull.* **1998**, *46*, 34.
27. Onaka, M.; Umezono, A.; Kawai, M.; Izumi, Y. *J. Chem. Soc., Chem. Commun.* **1985**, 1202.
28. Landais, Y.; Robin, J.-P.; Lebrun, A. *Tetrahedron* **1991**, *47*, 3787.
29. Barthel, W. F.; Alexander, B. H. *J. Org. Chem.* **1958**, *23*, 1012.
30. Albrecht, M. *Synthesis* **1996**, 230.
31. Razafimbelo, J.; Andriantsiferana, M.; Baudouin, G.; Tillequin, F. *Phytochemistry* **1996**, *41*, 323.
32. Bailly, C. *Methods Enzymol.* **2001**, *340*, 610.
33. Madelaine, J.; Prost, S.; Naudin, A.; Riou, G.; Lavelle, F.; Riou, J. F. *Biochem. Pharmacol.* **1993**, *45*, 339.
34. Pilch, B.; Allemand, E.; Facompré, M.; Bailly, C.; Riou, J. F.; Soret, J.; Tazi, J. *Cancer Res.* **2001**, *61*, 6876.
35. Bailly, C.; Lansiaux, A.; Dassonneville, L.; Demarquay, D.; Coulomb, H.; Huchet, M.; Lavergne, O.; Bigg, D. C. H. *Biochemistry* **1999**, *38*, 15556.
36. Colson, P.; Bailly, C.; Houssier, C. *Biophys. Chem.* **1996**, *58*, 125.
37. Bailly, C.; Hénichart, J. P.; Colson, P.; Houssier, C. *J. Mol. Recognit.* **1992**, *5*, 155.
38. Pierré, A.; Kraus-Berthier, L.; Atassi, Gh.; Cros, S.; Poupon, M. F.; Lavielle, G.; Berlion, M.; Bizarri, J. P. *Cancer Res.* **1991**, *51*, 2312.
39. Rossi, F.; Labourier, E.; Forné, T.; Divita, G.; Derancourt, J. F.; Antoine, E.; Cathala, G.; Brunel, C.; Tazi, J. *Nature* **1996**, *381*, 80.
40. Houssier, C. Investigating nucleic acids, nucleoproteins, polynucleotides, and their interactions with small ligands by electro-optical systems. In *Molecular Electro-Optics*; Krause, S., Ed.; Plenum: New York, 1981; pp 363–398.