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Synthesis, Cytotoxicity, DNA Interaction and Topoisomerase II Inhibition Properties of Tetrahydropyrrolo[3,4-*a*]carbazole-1,3-dione and Tetrahydropyrido-[3,2-*b*]pyrrolo[3,4-*g*]indole-1,3-dione Derivatives

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Abstract—Three tetrahydropyrrolo[3,4-*a*]carbazole-1,3-diones (**6**–**8**) and two tetrahydropyrido[3,2-*b*]pyrrolo[3,4-*g*]indole-1,3-diones (**11**–**12**) have been synthesized. Their interaction with DNA was probed by absorption and thermal melting studies. Compounds **8** and **12** both equipped with a hydroxyethyl–aminoethyl side-chain demonstrated higher affinities for poly(dA-dT)$_2$ than compounds **6**, **7** and **11** bearing a dimethylaminoethyl side-chain. Circular and electric linear dichroism measurements showed that all five drugs behave as typical DNA intercalating agents. A plasmid cleavage assay was used to evaluate the capacity of the drugs to inhibit human topoisomerase II. Compounds **8** and **12** which bind strongly to DNA were found to stabilize DNA-topoisomerase II covalent complexes but their topoisomerase II inhibitory properties do not correlate with their cytotoxic potential. Compounds **6** and **7** are essentially inactive whereas compounds **8**, **11** and **12** exhibit a high toxicity to P388 murine leukemia cells and provoke a marked accumulation in the G2/M phase of the cell cycle. These compounds form a new class of DNA-targeted antitumor agents.

Introduction

A large number of anticancer agents targeted to nucleic acids and inhibitors of DNA topoisomerases contains a planar chromophore substituted with one or two aminoalkyl side-chains.[1–6] This is the case for anthracycline antibiotics (doxorubicin), anthracenediones (mitoxantrone), some acridines and ellipticines, for example. The intercalation of planar aromatic molecules into the DNA double helix and poisoning of DNA topoisomerases I and/or II are considered to be important in the therapeutic action of many antitumor agents. The neutral-chromophore DNA intercalating drugs DACA (acridine-4-carboxamide), TAS-103 (indeno-quinoline) and NB-506 (glycosyl-indolocarbazole) are also built on this model. Their anti-topoisomerase properties are essential to their cytotoxic activities.[7–12]

Based on the structure of the indolocarbazole moiety of NB-506 and related compounds such as rebeccamycin and its tumor-active derivative J-107088,[13–16] we designed new tetracyclic neutral chromophores, tetrahydropyrrolo[3,4-*a*]carbazole-1,3-dione and tetrahydropyrido[3,2-*b*]pyrrolo[3,4-*g*]indole-1,3-diones, susceptible to intercalate into DNA and to interfere with topoisomerases.

Compounds **6**–**8** contain a carbazole nucleus adjacent to an imide ring analogous to that of NB-506 and J-107088. Compounds **6** and **7** differ by the presence or absence of a methyl group on the carbazole moiety but both contain a dimethyl–aminoethyl side-chain common to several DNA intercalators/topoisomerase II inhibitors, such as TAS-103, some ellipticines, lucanthone and the olivacine derivative S16020-2.[17,18] Another side chain, hydroxyethyl–aminoethyl, was grafted on the chromophore of compound **8**. In parallel, we also prepared the pyrido analogues of **7** and **8**. Compounds **11** and **12** contain the tetrahydropyrido[3,2-

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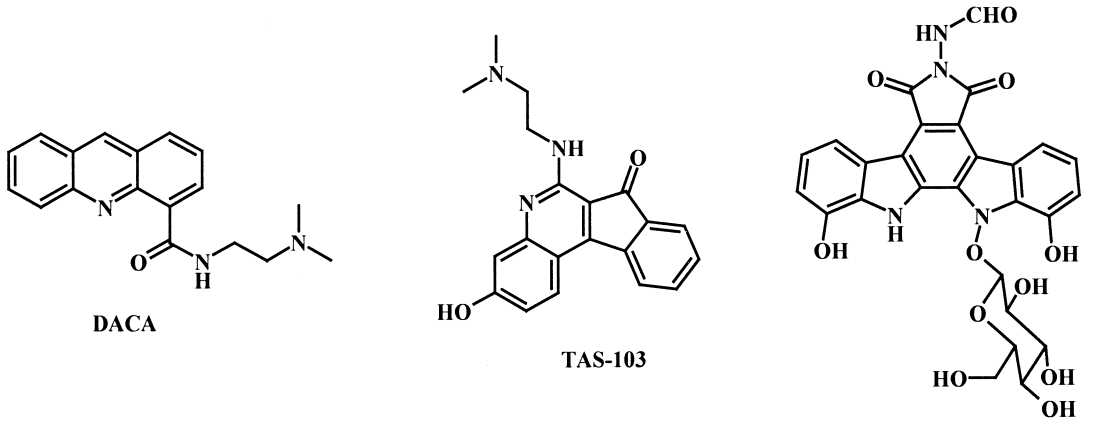
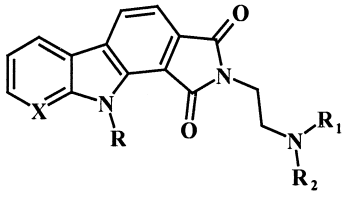




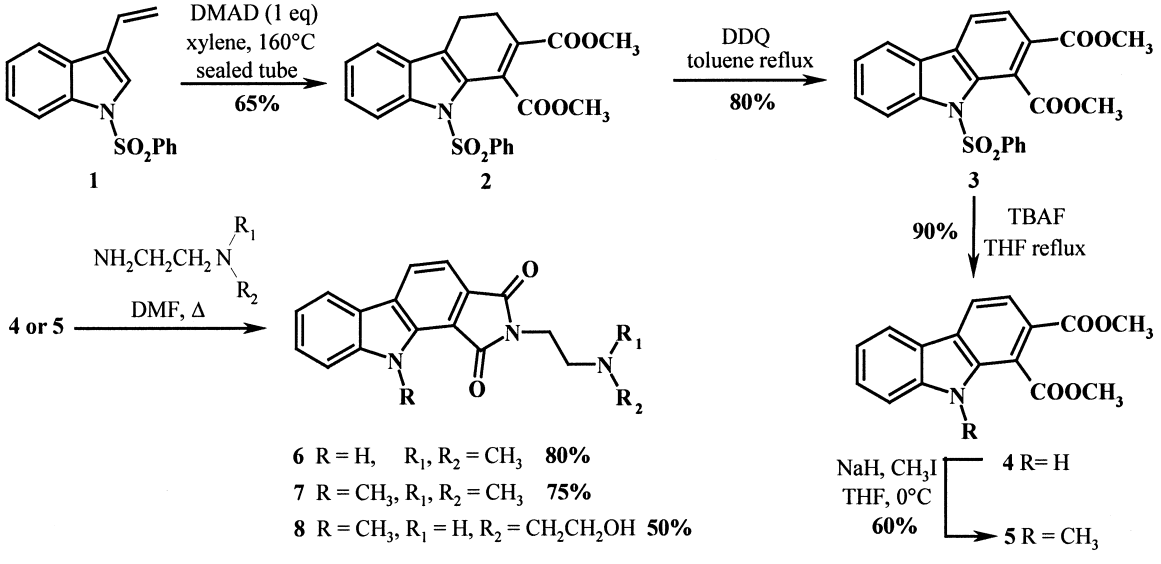
6 X = CH R = H $R_1 = CH_3$ $R_2 = CH_3$
7 X = CH R = CH_3 $R_1 = CH_3$ $R_2 = CH_3$
8 X = CH R = CH_3 R_1 = H $R_2 = CH_2CH_2OH$
11 X = N R = CH_3 $R_1 = CH_3$ $R_2 = CH_3$
12 X = N R = CH_3 R_1 = H $R_2 = CH_2CH_2OH$

b]pyrrolo[3,4-*g*]indole-1,3-dione nucleus substituted with one or the other of the two aforementioned side chains. In this paper, the synthesis of compounds **6–8**, **11–12** and their effects on DNA, human topoisomerases and P388 leukemia cells are presented.

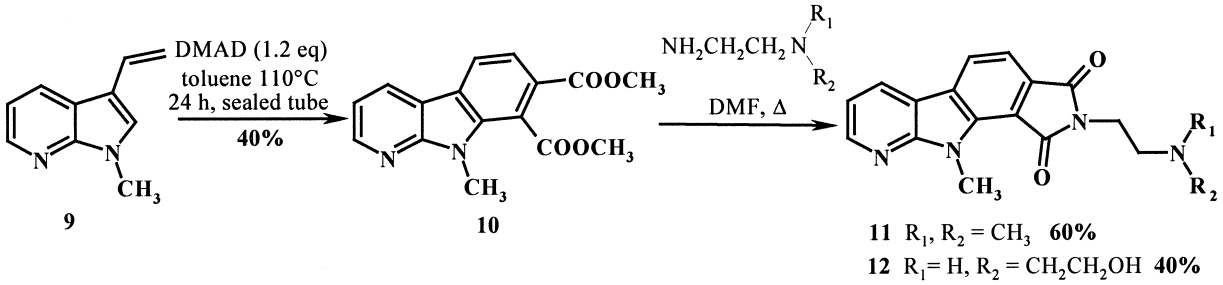
Results

Chemistry

The synthesis of target compounds **6–8** and **11–12** required the preparation of the key intermediates dimethyl 9-phenylsulfonyl-9*H*-1,2-carbazoledicarboxylate **3** and dimethyl 9-methyl-9*H*-pyrido[2,3-*b*]indole-7,8-dicarboxylate **10** (Schemes 1 and 2). Compounds **3** and **10** were obtained by Diels–Alder reaction[19,20] of 3-vinyl derivatives **1**[21] and **9**[20] with dimethyl acetylenedicarboxylate (DMAD). Cycloaddition reaction between **1** and DMAD in xylene led to the tetrahydrocarbazole **2** in 65% yield. The later product **3**[22–24] was prepared in 80% yield by oxidation of **2** with DDQ in refluxing toluene. Then, the *N*-phenylsulfonyl group was removed using tetrabutylammonium fluoride (TBAF)[25] in refluxing THF to afford **4** in 90% yield. *N*-Alkylation of **4** was carried out with iodomethane in THF at 0 °C in the presence of sodium hydride to give **5**[25] (60% yield). Finally, compounds **4** or **5** were treated



Scheme 1.



Scheme 2.

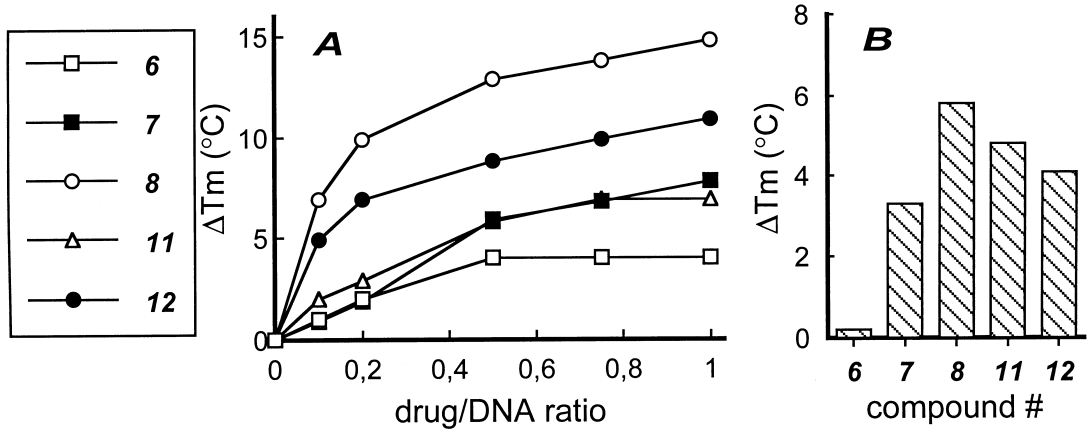


Figure 1. (A) Variation of the melting temperatures ($\Delta T_m = T_m$ complex$-T_m$ DNA) for the drugs bound to poly(dA-dT)•poly(dA-dT) as a function of the drug/DNA-phosphate (P/D) ratios. (B) Comparaison of the ΔT_m values measured for each compound bound to calf thymus DNA at a drug/DNA ratio of 1. T_m measurements were performed in BPE buffer pH 7.0 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA), in 1 cm quartz cuvettes at 260 nm with a heating rate of 1 °C/min. T_m values were obtained from first-derivative plots of the thermal denaturation curves.

with an excess of *N,N*-dimethylaminoethylamine or 2-(2-aminoethylamino)ethanol to afford expected compounds **6–8** in 50–80% yields.

Similarly, vinyl compound **9** was treated with DMAD (1.2 equiv) in toluene at 110 °C to give the dimethyl 9-methyl-9*H*-pyrido[2,3-*b*]indole-7,8-dicarboxylate **10** in 40% yield. As reported above, final derivatives **11–12** were prepared in 40–60% yields by treatment of **10** with amines.

DNA interaction

Melting temperatures, T_m, were measured for the compounds bound to poly(dA-dT)$_2$ to obtain a qualitative evaluation of the DNA binding affinity of these drug candidates. A complete set of experiments was performed at different drug/DNA ratio (Fig. 1A). The difference in T_m values (ΔTm) between the drug-polynucleotide complexes and free polynucleotide in solution provides a useful tool to assess the strength of the interaction of the molecules with DNA. The highest ΔT_m values were measured with compounds **8** and **12** both containing the hydroxyethyl–aminoethyl side-chain. For these two compounds, the ΔT_m values are comparable to those measured previously with glycosylated indolocarbazoles.[10] Lower values were obtained with the drugs having the dimethyl–aminoethyl side-chain. Compound **6** lacking the methyl group on the carbazole moiety was the less efficient compound in the series. However, this compound has significant interaction with DNA as judged from both the T_m measurements with poly(dA-dT)$_2$ and calf thymus DNA (which contains roughly equal proportions of A•T and G•C base pairs) (Fig. 1) and absorbance measurements. Addition of calf thymus DNA induced marked changes of the absorption spectrum of **6**: the intensity of the two absorption bands centered at 275 and 360 nm was strongly reduced while a new absorption band developed at 300 nm (Fig. 2). Similar spectral modifications were observed with all five drugs reflecting strong interaction between the electronic states of the drug chromophore and the DNA bases. From the T_m measurements, the relative binding affinities of the drugs for DNA rank in the order **8** > **12** > **7**,**11** > **6**.

The orientation of the ligands with respect to the DNA helix was determined by circular dichroism (CD) and electric linear dichroism (ELD) spectroscopy. The ELD spectra of compounds **6** and **12** bound to calf thymus DNA are shown in Figure 3A. In both cases, the reduced dichroism $\Delta A/A$ is negative in the drug absorption band which reflects the orientation of the chromophore along the electric field. The negative reduced dichroism values measured for each ligand bound to DNA (Fig. 3B) suggest that in all cases the chromophore is oriented parallel to the DNA base pairs, as expected for an intercalative binding. The intercalation binding mode is also supported by the CD measurements. A typical CD spectrum of compound **6** bound to DNA is presented in Figure 3C. A weak negative CD in the drug absorption region is commonly

observed with intercalating agents. Therefore, the two sets of spectroscopic measurements using polarized light, CD and ELD, are mutually consistent and indicate that the drugs intercalate into DNA.

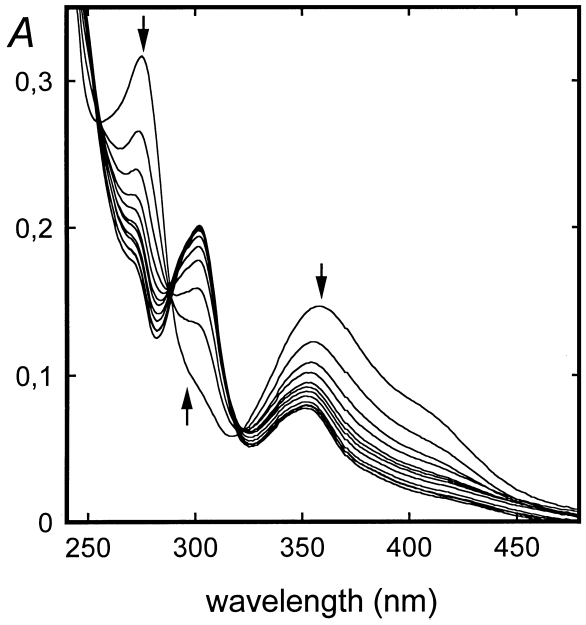


Figure 2. DNA titration of compound **6** in BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA) at pH 7.0. To 3 mL of drug solution at 20 μM were added aliquots of a concentrated calf thymus DNA solution. The phosphate-DNA/drug ratio increased from 0 to 20 (top to bottom curves, at 355 nm).

Topoisomerase II inhibition

A plasmid cleavage assay was used to investigate the effect of the drug candidates on human DNA topoisomerases I and II. For the topoisomerase I experiments, we found that the reference drug camptothecin produced a high level of nicked DNA molecules whereas the test molecules **6–8** and **11–12** were all totally inactive in this assay even at high concentrations (data not shown). For the topoisomerase II experiments, the reference drug was the epipodophyllotoxin etoposide which is a potent inhibitor stabilizing specifically DNA-topoisomerase II covalent complexes. In the presence of etoposide, topoisomerase II produces a substantial amount of linear DNA molecules. Interestingly compounds **8** and **12** also promoted the cleavage of DNA by the enzyme (Fig. 4). Therefore, these two molecules bearing the hydroxyethyl–aminoethyl side chain can be considered as topoisomerase poisons. Compounds **6**, **7** and **11** do not interfere with the cleavage activity of the enzyme. Compound **8** is slightly more efficient at stimulating DNA cleavage by topoisomerase II than compound **12**.

Cytotoxicity and cell cycle effects

The murine P388 leukemia cell line was used to assess the cytotoxic potential of this series. Using a conventional tetrazolium-based assay, we determined the drug concentrations required to inhibit cell growth by 50% after incubation in the culture medium for 72 h. The calculated IC_{50} values were 3.9, 1.9 and 1.0 μM for compounds **8**, **11** and **12**, respectively and IC_{50}'s were

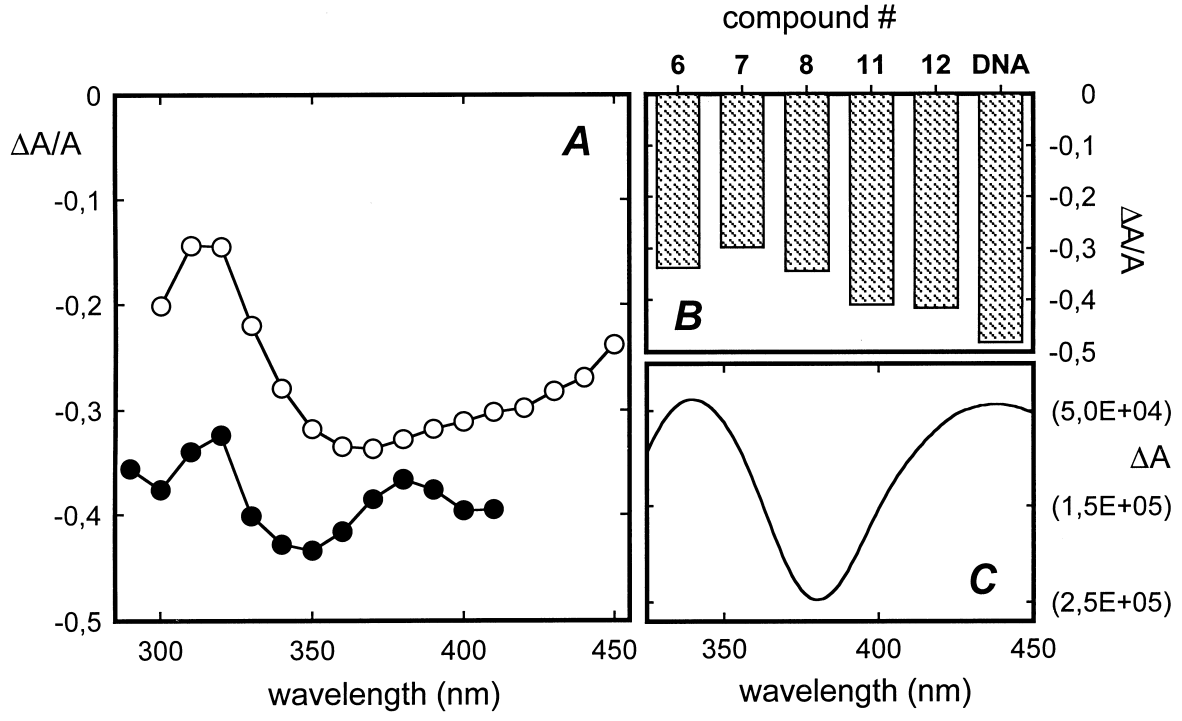


Figure 3. Circular and linear dichroism data. (A) Electric linear dichroism spectra of (○) compound **6** and (●) compound **12** bound to calf thymus DNA. (B) Reduced dichroism values (ΔA/A) measured with DNA alone at 260 nm and with the different drug-DNA complexes at 370 nm. Conditions: DNA-phosphate/drug (P/D) ratio of 20, electric field of 13.5 kV/cm. Panel C shows the induced circular dichroism spectrum of compound **6** bound to calf thymus DNA at a P/D = 50. All measurements were performed in 1 mM sodium cacodylate buffer, pH 7.0.

$>10\,\mu M$ for compounds **6** and **7**. Under the same experimental conditions, the control drugs camptothecin, TAS-103 and NB-506 gave IC_{50} values of 0.32, 0.005 and $0.24\,\mu M$, respectively.

Treatment of human HL-60 leukemia cells with increasing concentrations of compound **12** for 24 h led to important changes of the cell cycle profiles (Fig. 5). The flow cytometric analysis of propidium iodide-labeled cells indicates that the treatment with this drug at $10\,\mu M$ induces a massive accumulation of cells in the G2/M phase. The G2 cell population increases from 12% in the control to 61% in the presence of $10\,\mu M$ compound **12**. In the mean time, The G1-phase cell population gradually decreases from 56 to 4% and a significant proportion (20%) of cells with DNA content less than G1 was detected. The appearance of an important sub-G1 cell fraction suggests that this compound induces apoptosis, as is the case with conventional cytotoxic agents like etoposide or TAS-103.[26] In contrast, compound **6** produced no changes of the cell cycle profile (Fig. 5). This was the case also with compound **7**, in agreement with the cytotoxicity measurements mentioned above. P388 cells treated for 8 h with $10\,\mu M$ compounds **8**, **11** and **12** accumulated in the G2/M phase at 42, 50 and 61%, respectively.

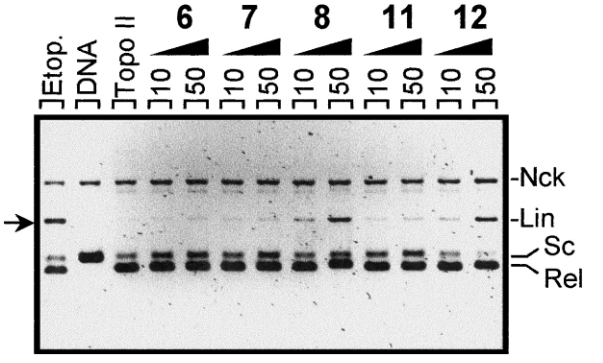


Figure 4. Effect of the drugs on the relaxation of plasmid DNA by human topoisomerase II. Native supercoiled pKMp27 DNA (0.5 μg) (lane DNA) was incubated with 4 units topoisomerase II in the absence (lane TopoII) or presence of drugs at the indicated concentration (μM). Etoposide (lane Etop.) was used at 50 μM. Reactions were stopped with sodium dodecylsulfate and treatment with proteinase K. DNA samples were separated by electrophoresis on an agarose gel containing ethidium bromide (1 μg/mL). The gel was photographed under UV light. Nck, nicked; Lin, linear; Rel, relaxed; Sc, supercoiled.

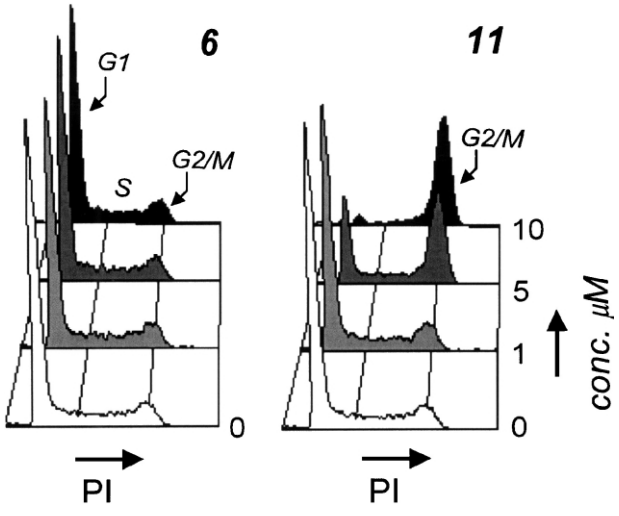


Figure 5. Cell cycle analysis of HL-60 cells treated with graded concentrations of compound **6** or compound **12** for 24 h. Cells were analyzed with a FACScan flow cytometer.

Discussion

As expected, the designed drugs are DNA intercalating agents. The strength of interaction with DNA is dependent on the nature of the side-chain. Compounds **8** and **12** bearing a hydroxyethyl–aminoethyl chain bind more strongly to DNA than those containing a dimethyl–aminoethyl side-chain. The hydroxyethyl–aminoethyl side-chain exists in different antineoplastic drugs including the anthracenedione mitoxantrone and the indenoisoquinoline drug oracin. This latter anticancer agent was found to induce G2 cell cycle arrest and apoptosis in Burkitt's lymphoma cells.[27] By analogy with related indenoisoquinoline derivatives, it is likely that oracin is a topoisomerase I poison.[28,29] The tetrahydropyrrolo[3,4-*a*]carbazole- and pyrido-indole-1,3-diones reported here do not inhibit topoisomerase I but two of them inhibit topoisomerase II. A link can be established between the DNA binding affinity and anti-topoisomerase II effect. Indeed, compounds **8** and **12** which both stimulate double-stranded DNA cleavage by the enzyme present a high affinity for DNA. These two compounds are highly cytotoxic and induce G2 cell cycle arrest in leukemia cells. One can imagine that their cytotoxic potential may derive from their capacity to bind to DNA and to inhibit topoisomerase II. However, this may not be necessary the case because compound **11** which has a modest affinity for DNA and is not a topoisomerase II poison, is also a highly cytotoxic agent. Based on the present results, compound **12** appears as a potential candidate for further evaluation in vivo as an anticancer agent.

Experimental

Chemistry

Melting points were determined using a Büchi capillary instrument and are uncorrected. IR spectra were recorded on a Perkin Elmer FTIR Paragon 1000 spectrophotometer. NMR spectra were recorded at 300 K on a Bruker Avance DPX 250 spectrometer. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS) and spin multiplicities are given as s (singlet), br s (broad singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Mass spectra were recorded on Perkin-Elmer SCIEX API 300 instrument using ion spray methodology. Column chromatography was performed on Merck silica gel (230-400 mesh). Analytical thin-layer chromatography (TLC) was run on precoated silica gel plates (Merck $60F_{254}$) and visualised with a UV light at 254 nm. Most chemicals and solvents were analytical grade and used without further purification.

Dimethyl 9-phenylsulfonyl-4,9-dihydro-*3H*-1,2-carbazoledicarboxylate (2). A solution of compound **1** (822 mg, 2.90 mmol) and dimethyl acetylenedicarboxylate (0.36 mL, 2.93 mmol) in xylene (10 mL) in a sealed tube was heated to 160 °C for 24 h. After cooling, the solvent was removed in vacuo. The crude residue was purified by column chromatography (eluent petroleum ether/ethyl acetate 7:3) to afford **2** (801 mg, 65%) as white crystals; mp 171–173 °C (Ethyl acetate/petroleum ether); IR (KBr) ν 1736, 1708 (CO) cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 2.58–2.76 (m, 4H, CH_2), 3.76 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 7.28 (t, 1H, $J=7.5$ Hz, H_{Ar}), 7.37–7.61 (m, 7H, H_{Ar}), 7.93 (d, 1H, $J=8.0$ Hz, H_{Ar}); ^{13}C NMR (62.90 MHz, DMSO-d_6) δ 19.2 (CH_2), 25.4 (CH_2), 52.1 (CH_3), 52.3 (CH_3), 116.1 (CH), 120.3 (CH), 125.2 (C), 126.3 (CH), 126.9 (2 CH), 127.6 (C), 128.9 (C), 129.3 (3 CH), 132.0 (C), 133.0 (C), 134.5 (CH), 134.6 (C), 138.2 (C), 165.1 (CO), 167.8 (CO). Anal. calcd for $C_{22}H_{19}NO_6S$: C, 62.11; H, 4.50; N, 3.29. Found: C, 62.45; H, 4.68; N, 3.20; MS m/z 426 $(M+1)^+$.

Dimethyl 9-phenylsulfonyl-9*H*-1,2-carbazoledicarboxylate (3). A solution of **2** (422 mg, 0.10 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (680 mg, 0.30 mmol) in dry toluene (20 mL) was stirred at reflux under argon for 24 h. After cooling, the solution was diluted with ethyl acetate (20 mL), then washed successively with 10% sodium hydroxide (twice) and water. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The crude residue was purified by column chromatography (eluent petroleum ether/ethyl acetate 6:4) to afford **3** (338 mg, 80%) as white crystals; mp 168–170 °C (ethyl acetate) (lit.[22] mp 175 °C); IR (KBr) ν 1734, 1725 (CO) cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 3.88 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 7.23–7.34 (m, 4H, H_{Ar}), 7.42–7.64 (m, 3H, H_{Ar}), 7.83 (d, 1H, $J=8.0$ Hz, H_{Ar}), 7.81 (d, 1H, $J=8.0$ Hz, H_{Ar}), 7.88 (d, 1H, $J=8.0$ Hz, H_{Ar}), 8.29 (d, 1H, $J=8.0$ Hz, H_{Ar}); ^{13}C NMR (62.90 MHz, DMSO-d_6) δ 52.5 (CH_3), 52.9 (CH_3), 117.7 (CH), 121.4 (CH), 122.5 (CH), 124.3 (C), 126.1 (CH), 126.3 (2 CH), 126.4 (C), 126.5 (CH), 129.1 (2 CH), 129.3 (CH), 130.3 (C), 132.4 (C), 134.5 (C), 134.6 (CH), 135.3 (C), 140.2 (C), 165.9 (CO), 167.4 (CO). Anal. calcd for $C_{22}H_{17}NO_6S$: C, 62.40; H, 4.05; N, 3.31. Found: C, 62.64; H, 4.22; N, 3.50; MS m/z 424 $(M+1)^+$.

Dimethyl 9*H*-1,2-carbazoledicarboxylate (4). A solution of **3** (424 mg, 1.00 mmol) and freshly prepared tetrabutylammonium fluoride (392 mg, 1.50 mmol) in dry THF (20 mL) was stirred at reflux under argon for 2 h. After cooling, THF was removed in vacuo. The residue was partitioned between ethyl acetate (10 mL) and water (10 mL), the aqueous phase separated and extracted with ethyl acetate (2×5 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude oil was purified by column chromatography (eluent petroleum ether/ethyl acetate 6:4) to afford **4** (283 mg, 100%) as white crystals; mp 84–86 °C (ethyl acetate); IR (KBr) ν 3411 (NH), 1715 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.95 (s, 3H, CH_3), 3.98 (s, 3H, CH_3), 7.21–7.28 (m, 1H, H_{Ar}), 7.33 (d, 1H, $J=8.0$ Hz, H_{Ar}), 7.44–7.47 (m, 2H, H_{Ar}), 8.07 (br d, 1H, $J=7.8$ Hz, H_{Ar}), 8.19 (br d, 1H, $J=7.8$ Hz, H_{Ar}), 9.72 (br s, 1H, NH); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ 52.6 (CH_3), 52.8 (CH_3), 110.2 (C), 111.3 (CH), 118.7 (CH), 120.4 (CH), 120.9 (CH), 122.0 (C), 124.1 (CH), 126.3 (C), 127.5 (CH), 131.9 (C), 139.3 (C), 140.5 (C), 167.0 (CO), 170.2 (CO). Anal. calcd for $C_{16}H_{13}NO_4$: C, 67.84; H, 4.63; N, 4.94. Found: C, 67.53; H, 4.47; N, 5.04; MS m/z 284 $(M+1)^+$.

Dimethyl 9-methyl-9*H*-1,2-carbazoledicarboxylate (5). To a solution of **4** (207 mg, 0.73 mmol) in dry THF (10 mL) under argon was added slowly sodium hydride (30 mg, 1.10 mmol) at 0 °C for 30 min. After 30 min of stirring, a solution of iodomethane (67 μL, 1.10 mmol) in THF (5 mL) was added. The final solution was stirred at 0 °C for 1 h, hydrolyzed with water (5 mL) and finally THF was removed in vacuo. The residue was partitioned between ethyl acetate (10 mL) and water (10 mL), the aqueous phase separated and extracted with ethyl acetate (2×10 mL). The organic phase was dried over $MgSO_4$ and evaporated in vacuo. The crude residue was purified by column chromatography (eluent petroleum ether/ethyl acetate 7:3) to give **5** (130 mg, 60%) as white crystals; mp 120–121 °C (petroleum ether–ethyl acetate) (lit.[19] mp 122.5–124 °C); IR (KBr) ν 1741, 1715 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.85 (s, 3H, CH_3), 3.95 (s, 3H, CH_3), 4.08 (s, 3H, CH_3), 7.28 (t, 1H, $J=8.0$ Hz, H_{Ar}), 7.42 (d, 1H, $J=8.0$ Hz, H_{Ar}), 7.56 (t, 1H, $J=8.0$ Hz, H_{Ar}), 7.90 (d, 1H, $J=8.0$ Hz, H_{Ar}), 8.11 (d, 1H, $J=8.0$ Hz, H_{Ar}), 8.15 (d, 1H, $J=8.0$ Hz, H_{Ar}); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ 30.4 (CH_3), 52.6 (CH_3), 53.0 (CH_3), 109.2 (CH), 118.9 (C), 120.0 (CH), 120.5 (CH), 120.7 (CH), 121.1 (CH), 121.4 (C), 124.6 (C), 127.9 (CH), 128.1 (C), 136.4 (C), 143.0 (C), 167.1 (CO), 169.8 (CO). Anal. calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 69.04; H, 5.21; N, 4.60; MS m/z 298 $(M+1)^+$.

2-[2-(Dimethylamino)ethyl]-1,2,3,10-tetrahydropyrrolo-[3,4-*a*]carbazole-1,3-dione (6). A solution of **4** (71 mg, 0.25 mmol) in *N,N*-dimethylethylenediamine (3 mL) and *N,N*-dimethylformamide (3 mL) was stirred at 130 °C for 24 h. After cooling and evaporation of solvent, the crude residue was crystallized from methanol to give **6** (62 mg, 80%) as yellow crystals; mp 192–193 °C (methanol); IR (KBr) ν 1757, 1701 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 2.55 (s, 6H, CH_3), 2.94 (t, 2H, $J=6.7$ Hz, CH_2), 3.88 (t, 2H, $J=6.7$ Hz, CH_2), 6.80 (d, 1H, $J=7.7$ Hz, H_{Ar}), 7.29 (t, 1H, $J=8.0$ Hz, H_{Ar}), 7.46 (d, 1H, $J=8.0$ Hz, H_{Ar}), 7.55 (t, 1H, $J=8.0$ Hz, H_{Ar}), 7.79 (d, 1H, $J=7.7$ Hz, H_{Ar}), 7.94 (d, 1H, $J=8.0$ Hz, H_{Ar}), 10.55 (br s, 1H, NH); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ 35.5 (CH_2), 45.9 (2 CH_3), 57.8 (CH_2), 112.2 (CH), 112.9 (C), 113.1 (CH), 120.6 (CH), 120.7 (CH), 121.9 (C), 124.7 (CH), 128.0 (CH), 128.7 (C), 129.9 (C), 133.2 (C), 142.0 (C), 168.7 (CO), 169.2 (CO). Anal. calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67. Found: C, 69.98; H, 5.45; N, 13.80; MS m/z 308 $(M+1)^+$.

2-[2-(Dimethylamino)ethyl]-10-methyl-1,2,3,10-tetrahydropyrrolo[3,4-*a*]carbazole-1,3-dione (7). Following the procedure used for the preparation of **6**, compound **5** in

the presence of *N,N*-dimethylethylenediamine gave **7** in 75% yield as yellow crystals; mp 218–219 °C (methanol); IR (KBr) ν 1753, 1698 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 2.33 (s, 6H, CH_3), 2.64 (t, 2H, $J=6.7$ Hz, CH_2), 3.85 (t, 2H, $J=6.7$ Hz, CH_2), 4.45 (s, 3H, CH_3), 7.33 (t, 1H, $J=8.0$ Hz, H_{Ar}), 7.48 (d, 1H, $J=8.0$ Hz, H_{Ar}), 7.60 (d, 1H, $J=8.0$ Hz, H_{Ar}), 7.68 (d, 1H, $J=7.7$ Hz, H_{Ar}), 8.12 (d, 1H, $J=8.0$ Hz, H_{Ar}), 8.33 (d, 1H, $J=7.7$ Hz, H_{Ar}); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ 33.6 (CH_3), 36.1 (CH_2), 45.7 (2 CH_3), 57.5 (CH_2), 109.8 (CH), 113.3 (C), 113.6 (CH), 120.7 (CH), 120.8 (CH), 122.2 (C), 125.3 (CH), 128.2 (CH), 130.2 (C), 130.9 (C), 136.9 (C), 143.2 (C), 168.7 (CO), 169.3 (CO). Anal. calcd for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.07. Found: C, 70.81; H, 6.12; N, 12.88; MS *m/z* 322 $(M+1)^+$.

2-{2-[(2-Hydroxyethyl)amino]ethyl}-10-methyl-1,2,3,10-tetrahydropyrrolo[3,4-*a*]carbazole-1,3-dione (8). Following the procedure used for the preparation of **7**, compound **5** in the presence of 2-(2-aminoethylamino)ethanol gave **8** (50% yield) as yellow crystals after purification by column chromatography (eluent dichloromethane/methanol 9:1); mp 158–159 °C (methanol); IR (KBr) ν 3600–3000 (OH), 3326 (NH), 1753, 1700 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3+D_2O$) δ 2.87 (t, 2H, $J=5.2$ Hz, CH_2), 3.01 (t, 2H, $J=6.0$ Hz, CH_2), 3.66 (t, 2H, $J=5.2$ Hz, CH_2), 3.86 (t, 2H, $J=6.0$ Hz, CH_2), 4.36 (s, 3H, CH_3), 7.31 (t, 1H, $J=8.0$ Hz, H_{Ar}), 7.42 (d, 1H, $J=8.0$ Hz, H_{Ar}), 7.57 (t, 1H, $J=8.0$ Hz, H_{Ar}), 7.61 (d, 1H, $J=7.7$ Hz, H_{Ar}), 8.05 (d, 1H, $J=8.0$ Hz, H_{Ar}), 8.25 (d, 1H, $J=7.7$ Hz, H_{Ar}); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ 33.5 (CH_3), 37.8 (CH_2), 47.8 (CH_2), 50.8 (CH_2), 60.9 (CH_2), 109.8 (CH), 112.9 (C), 113.6 (CH), 120.8 (2 CH), 122.1 (C), 125.4 (CH), 128.3 (CH), 130.3 (C), 130.6 (C), 136.8 (C), 143.1 (C), 168.9 (CO), 169.5 (CO). Anal. calcd for $C_{19}H_{19}N_3O_3$: C, 67.64; H, 5.68; N, 12.45. Found: C, 67.99; H, 5.55; N, 12.62; MS *m/z* 338 $(M+1)^+$.

Dimethyl 9-Methyl-9*H*-pyrido[2,3-*b*]indole-7,8-dicarboxylate (10). A solution of compound **9** (190 mg, 0.97 mmol) and dimethyl acetylenedicarboxylate (0.18 mL, 1.46 mmol) in toluene (10 mL) in a sealed tube was heated at 110 °C for 24 h. After cooling, the solvent was removed in vacuo. The crude residue was purified by column chromatography (eluent petroleum ether/ethyl acetate 7:3) to afford **10** (143 mg, 40%) as white crystals; mp 151–152 °C (methanol); IR (KBr) ν 1736, 1710 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.96 (s, 3H, CH_3), 3.99 (s, 3H, CH_3), 4.10 (s, 3H, CH_3), 7.21 (dd, 1H, $J=4.8$, 7.5 Hz, H_{Pyr}), 7.92 (d, 1H, $J=7.2$ Hz, H_{Ar}), 8.11 (d, 1H, $J=7.2$ Hz, H_{Ar}), 8.33 (dd, 1H, $J=1.5$, 7.5 Hz, H_{Pyr}), 8.58 (dd, 1H, $J=1.5$, 4.8 Hz, H_{Pyr}); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ 28.4 (CH_3), 52.6 (CH_3), 52.9 (CH_3), 114.2 (C), 115.9 (CH), 119.3 (C), 121.0 (CH + C), 121.3 (CH), 125.3 (C), 129.0 (CH), 135.5 (C), 148.0 (CH), 152.8 (C), 166.5 (CO), 169.1 (CO). Anal. calcd for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.07; H, 4.81; N, 9.50; MS *m/z* 299 $(M+1)^+$.

2-[2-(Dimethylamino)ethyl]-6-methyl-1,2,3,6-tetrahydropyrido[2,3-*b*]pyrrolo[3,4-*e*]- indole-1,3-dione (11). Following the procedure used for the preparation of **6**, compound **10** in the presence of *N,N*-dimethylethylenediamine gave **11** in 60% yield as yellow crystals; mp 189–190 °C (methanol); IR (KBr) ν 1762, 1698 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 2.33 (s, 6H, CH_3), 2.65 (t, 2H, $J=6.7$ Hz, CH_2), 3.87 (t, 2H, $J=6.7$ Hz, CH_2), 4.51 (s, 3H, CH_3), 7.28 (dd, 1H, $J=5.0$, 8.2 Hz, H_{Pyr}), 7.73 (d, 1H, $J=7.7$ Hz, H_{Ar}), 8.34 (d, 1H, $J=7.7$ Hz, H_{Ar}), 8.37 (dd, 1H, $J=1.5$, 8.2 Hz, H_{Pyr}), 8.64 (dd, 1H, $J=1.5$, 5.0 Hz, H_{Pyr}); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ 32.1 (CH_3), 36.0 (CH_2), 45.6 (2 CH_3), 57.2 (CH_2), 114.1 (CH + C), 114.8 (C), 116.5 (CH), 125.9 (CH), 127.2 (C), 128.8 (CH), 131.5 (C), 135.8 (C), 148.3 (CH), 153.2 (C), 167.9 (CO), 168.8 (CO). Anal. calcd for $C_{18}H_{18}N_4O_2$: C, 67.07; H, 5.63; N, 17.38. Found: C, 66.81; H, 5.70; N, 17.25; MS *m/z* 323 $(M+1)^+$.

2-{2-[(2-Hydroxyethyl)amino]ethyl}-10-methyl-1,2,3,10-[2,3-*b*]pyrrolo[3,4-*e*]indole-1,3-dione (12). Following the procedure used for the preparation of **8**, compound **10** in the presence of 2-(2-aminoethylamino)ethanol gave **12** (40% yield) as yellow crystals after purification by column chromatography (eluent dichloromethane/methanol 9:1); mp 184–185 °C (methanol); IR (KBr) ν 3600–3000 (OH), 3295 (NH), 1755, 1699 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3+D_2O$) δ 2.87 (t, 2H, $J=5.2$ Hz, CH_2), 3.01 (t, 2H, $J=6.0$ Hz, CH_2), 3.63 (t, 2H, $J=5.2$ Hz, CH_2), 3.88 (t, 2H, $J=6.0$ Hz, CH_2), 4.50 (s, 3H, CH_3), 7.29 (dd, 1H, $J=5.0$, 8.2 Hz, H_{Pyr}), 7.75 (d, 1H, $J=7.5$ Hz, H_{Ar}), 8.35 (d, 1H, $J=7.5$ Hz, H_{Ar}), 8.38 (dd, 1H, $J=1.5$, 8.2 Hz, H_{Pyr}), 8.64 (dd, 1H, $J=1.5$, 5.0 Hz, H_{Pyr}); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ 32.3 (CH_3), 38.1 (CH_2), 47.8 (CH_2), 50.9 (CH_2), 61.2 (CH_2), 114.4 (CH + C), 115.1 (C), 116.7 (CH), 126.3 (CH), 127.6 (C), 129.1 (CH), 131.5 (C), 136.0 (C), 148.6 (CH), 153.4 (C), 168.3 (CO), 169.3 (CO). Anal. calcd for $C_{18}H_{18}N_4O_3$: C, 63.89; H, 5.36; N, 16.56. Found: C, 64.10; H, 5.42; N, 16.76; MS *m/z* 339 $(M+1)^+$.

Chemicals and biochemicals

Etoposide, camptothecin, calf thymus DNA and the double-stranded polymer poly(dA-dT)$_2$ were purchased from Sigma Chemical Co. (La Verpillière, France). Calf thymus DNA was deproteinized with sodium dodecyl sulphate. All other chemicals were analytical grade reagents.

Absorption spectra and melting temperature studies

Melting curves were measured using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. For each series of measurements, 12 samples were placed in a thermostatically controlled cell-holder, and the quartz cuvettes (10 mm pathlength) were heated by circulating water. Measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA). The temperature inside the cuvette was measured with a platinum probe; it was increased over the range 20–100 °C with a heating rate of 1 °C/min. The "melting" temperature T_m was taken as the midpoint of the hyperchromic transition. The Uvikon 943 spectrophotometer was also used to record the

absorption spectra. 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). DNA blanks at the same nucleotide concentrations were prepared concomitantly and used as a reference in the recording of absorption spectra.

Circular dichroism

CD measurements were recorded on a Jobin-Yvon CD6 dichrograph. Solutions of drugs and/or nucleic acids were scanned in a 2 cm-quartz cuvette, in 1 mM sodium cacodylate buffer, pH 7.0. Measurements were made by progressive addition of DNA to a pure ligand solution to obtain the desired drug/nucleic acid ratios. Three scans were accumulated and automatically averaged.

Electric linear dichroism

ELD measurements were performed with a computerized optical measurement system using the procedures previously outlined.[30] 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 13 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.[31]

Topoisomerase-mediated DNA cleavage assay

Supercoiled pKMp27 DNA (0.5 μg) was incubated with 4 units human topoisomerase I or II (TopoGen Inc.) at 37 °C for 30 min in relaxation buffer (50 mM Tris pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA and ATP) 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 a 1% agarose gel containing ethidium bromide (1 μg/mL), at room temperature for 2 h at 120V. Gels were washed and photographed under UV light.

Cell cultures and survival assay

The P388 murine leukaemia cell line was kindly provided by Dr. J.-F. Riou (Rhône-Poulenc Rorer, France). Human HL-60 promyelocytic leukemia cells were obtained from the American Tissue Culture Collection (Manassas, VA). Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in RPMI 1640 medium, supplemented with 10% fetal bovine serum, glutamine (2 mM), penicillin (100 IU/mL) and streptomycin (100 μg/mL). The cytotoxicity of the test compounds was assessed using a cell proliferation assay developed by Promega (CellTiter 96® AQ_{ueous} one solution cell proliferation assay). Briefly, 2×10^4 exponentially growing cells were seeded in 96-well microculture plates with various drug concentrations in a volume of 100 μL. After 72 h incubation at 37 °C, 20 μL of MTS[32] were added to each well and the samples were incubated for a further 3 h at 37 °C. Plates were analyzed on a Labsystems Multiskan MS (type 352) reader at 492 nm.

Cell cycle analysis

For flow cytometry analysis of DNA content, 10^6 HL-60 cells in exponential growth were treated with graded concentrations of the test drug for 24 h and then washed 3 times with citrate buffer. The cell pellet was incubated with 250 μL of trypsin-containing citrate buffer for 10 min at room temperature and then with 200 μL of citrate buffer containing a trypsin inhibitor and RNase (10 min) prior to adding 200 μL of propidium iodide (PI) at 125 μg/mL. Samples were analyzed on a Becton Dickinson FACScan flow cytometer using the LYSYS II software which is also used to determine the percentage of cells in the different phases of the cell cycle. PI was excited at 488 nm, and fluorescence analyzed at 620 nm (Fl-3).

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