Synthesis of Mono- and Bisdihydrodipyridopyrazines and Assessment of Their DNA Binding and Cytotoxic Properties

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Aminoalkyl-substituted monomeric and dimeric dihydrodipyridopyrazines have been synthesized and evaluated as antitumor agents. Potent cytotoxic compounds were identified in both series. Biochemical and biophysical studies indicated that all these compounds strongly stabilized the duplex structure of DNA and some of them elicited a selectivity for GC-rich sequences. Sequence recognition by of the dimeric dihydrodipyridopyrazines is reminiscent of that of certain antitumor bisnaphthalimides. Compared to monomers, corresponding dimeric derivatives showed higher affinity for DNA. This property was attributed to a bisintercalative binding to DNA. This assumption was indirectly probed by electric linear dichroism and DNA relaxation experiments. DNA provides a bioreceptor for these dihydrodipyridopyrazine derivatives, but no poisoning of human topoisomerases I or II was detected. Most of the compounds efficiently inhibited the growth of L1210 murine leukemia cells and perturbed the cell cycle progression (with a G2/M block in most cases). A weak but noticeable in vivo antitumor activity was observed with one of the dimeric compounds. This studies identifies monomeric and dimeric dihydrodipyridopyrazines as a new class of DNA-targeted antitumor agents.

Introduction

The design of DNA bisintercalators can be traced back to the early to mid-1970s when the groups of Le Pecq in France and Cain in New Zealand pioneered the synthesis of acridine dimers incorporating aminoalkyl linkers of variable lengths.[1] Over the past 30 years a large diversity of dimeric forms of DNA intercalators has been developed as potential anticancer drugs. One can refer to bisphenanthridines, bisacridinecarboxamides, bisnaphthalimides, bisphenazinecarboxamides, and bisimidazoacridones, to cite only a few of the dimeric antitumor agents recently elaborated. Many of these compounds were reported to function as DNA-targeted topoisomerase I and/or II inhibitors. The promising in vivo activity of drugs such as bisnafide (bisnaphthalimide),[2] ditercalinium (bispyridocarbazole),[3] WP631 (bisanthracyclines),[4] and WMC26 (bisimidazoacridone)[5] (Chart 1) has stimulated the search for novel bifunctional DNA ligands. In general, the basic idea behind the design of dimers is to increase DNA binding affinity and sequence recognition. However, in some cases the second chromophore does not play the expected role of a DNA intercalator but it can serve as a hook to trap DNA binding proteins or their associated cofactors.

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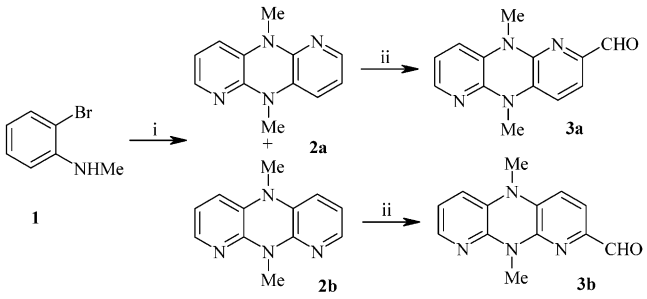
Scheme 1[a]



[a] (i) $NaNH_2$-*t*-BuONa, THF; (ii) $POCl_3$, DMF, then KOH.

Recently, we developed dihydrodipyridopyrazines as a new family of potential antitumor agents.[6] By analogy with related structures (e.g., phenazine and acridines), these tricyclic planar molecules are thought to intercalate into DNA and might interfere with topoisomerase activities. To extend the structure−activity relationships in this series, we report here the synthesis of a variety of aminoalkyl-substituted monomeric and dimeric dihydrodipyridopyrazines, together with information on their mechanism of action.

Results

Synthesis. The procedure for the synthesis of aldehyde **3** (Scheme 1) has been previously described.[7] Briefly, arynic cyclization of **1** in the presence of the complex base[8] $NaNH_2$-*t*-BuONa led to the simultaneous formation of **2a** and **2b**, which were easily separated

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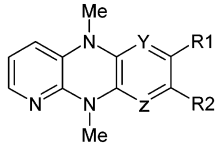
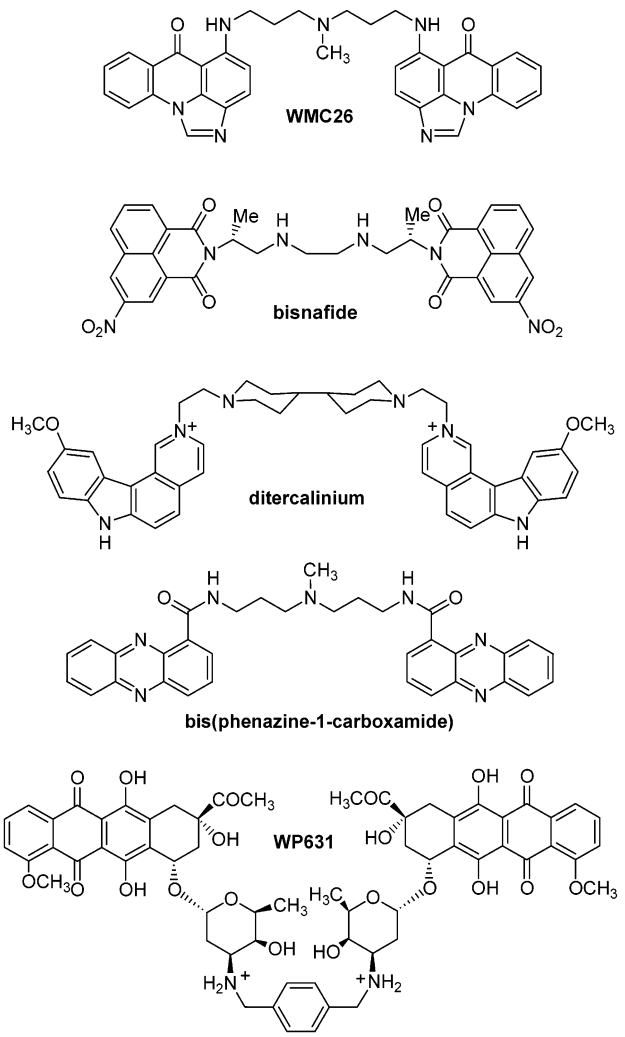
Table 1. Synthesis and Cytotoxic Evaluation of Selected Dihydrodipyridopyrazine Derivatives **2**−**11**, **14**, and **15**



compd	Y	Z	R^1	R^2	yield (%)	IC_{50} (μM)	cycle[a]
2a	N	CH	H	H	25[b]	31.1	51% S at 100 μM
2b	CH	N	H	H	25[b]	34.0	49% S, 34% G2M at 100 μM
3a	N	CH	CHO	H	30[b]	21.6	90% G2M at 50 μM
3b	CH	N	H	CHO	80[b]	20	ND[c]
4	N	CH	$CH_2NH(CH_2)_2NMe_2$	H	80[d]	26.7	55% G1 at 25 μM
5	N	CH	$CH_2NH(CH_2)_3NMe_2$	H	91[d]	9.2	39% G2M at 25 μM
6	CH	N	H	$CH_2NH(CH_2)_2NMe_2$	75[d]	11.3	47% G2M, 4% 8N at 10 μM[e]
7	CH	N	H	$CH_2NH(CH_2)_3NMe_2$	91[d]	0.17	66% G2M, 14% 8N at 1 μM
8	CH	N	H	$CH_2NH(CH_2)_4NMe_2$	86[d]	0.14	54% G2M, 32% 8N at 0.5 μM
9	CH	N	H	$CH_2NH(CH_2)_5NMe_2$	83[d]	0.5	toxic, NS[f]
10	CH	N	H	$CH_2NH(CH_2)_3NH_2$	60[d]	0.14	65% G2M 8N at 1 μM (toxic)
11	CH	N	H	$CH_2N(Me)$-$(CH_2)_3$-NMe_2	62[d]	11.5	ND
14	CH	N	H	CH_2-$NHCO$-$(CH_2)_2$-NMe_2	35[g]	21.1	ND
15	CH	N	H	$CONH$-$(CH_2)_3$-NMe_2	35[d]	1.7	67% G2M at 10 μM

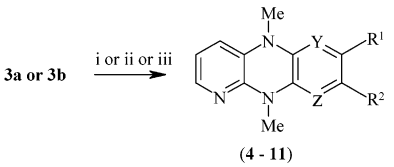
[a] Initially 42%, 34%, and 24% of untreated L1210 cells were in G1, S, and G2M phases of the cell cycle. [b] Obtained according to ref 7. [c] Not determined. [d] With respect to **3**. [e] Toxic at 25 μM. [f] No specific effect. [g] With respect to **13**.

Chart 1: Structure of Selected DNA Binding Dimers



by chromatography. Vilsmeier reaction performed with each of the isomers produced the desired starting materials **3a** and **3b**. These compounds were found to be only modestly cytotoxic toward L1210 murine leukemia cells with IC_{50} values in the 20−30 μM range

Scheme 2[a]

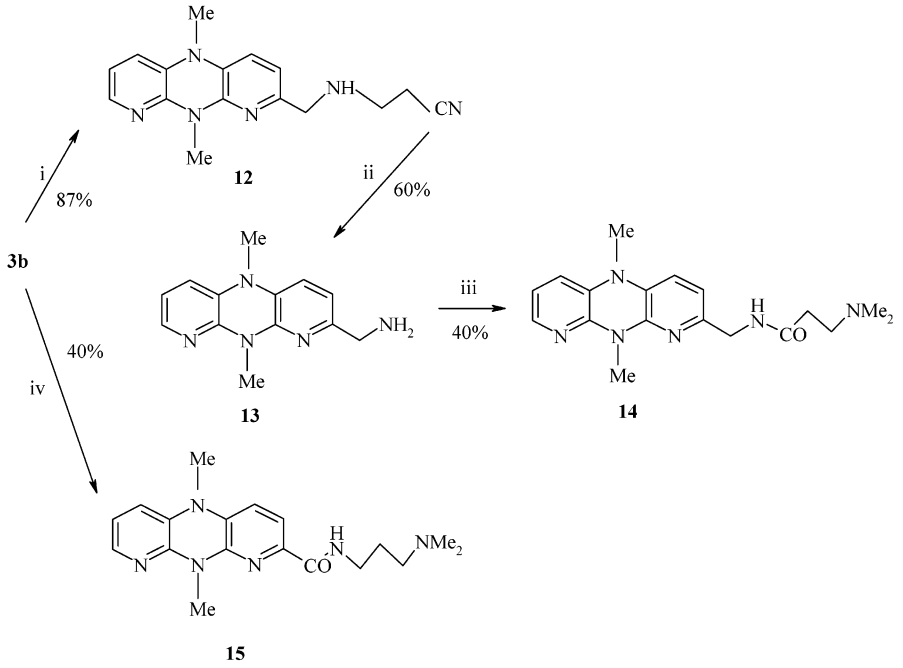


[a] (i) (1) H_2N-$(CH_2)_n$-NMe_2, $n = 2-5$, CH_2Cl_2; (2) $NaBH_4$, MeOH. (ii) (1) H_2N-$(CH_2)_3$-$NHCOO$-Bu^t, CH_2Cl_2; (2) $NaBH_4$, MeOH; (3) HCOOH. (iii) (1) H_2N-$(CH_2)_3$-NMe_2, CH_2Cl_2; (2) $NaBH_4$, MeOH; (3) formalin, MeOH; (4) $NaBH_4$, MeOH.

(Table 1). Considering that the incorporation of an aminoalkyl substituent generally enhances the cytotoxic potential of tri- and tetracyclic DNA binding agents,[9] it was then decided to introduce such amino groups on **3a** and **3b**, as depicted in Scheme 2. Compounds **4**−**9** were obtained in good yields by classical reductive amination. The product **10** necessitated the amination by the monoprotected 1,3-diaminopropane, followed by the release of the primary amino group. The methylated compound **11** was obtained from **7** by reaction with formalin and reduction of the intermediate thus prepared.

Previous studies with acridine and phenazine derivatives have shown that the replacement of an aminomethyl substituent by an amido or methylamido group on an aryl or heteroaryl ring frequently promotes the anticancer activity of the compounds.[10] For this reason, the amido analogue **15** of the highly cytotoxic compound **7** (Table 1) was prepared. This was achieved from **3b** as indicated in Scheme 3. Reductive aminations performed to prepare the intermediate **13** were unsuccessful or gave very poor yields. Therefore, it was decided to adopt an alternate route with an indirect sequence via **12** (a compound initially prepared for other investigations), easily obtained by classical reductive amination with 3-aminopropionitrile. Incidentally, we found that warming **12** at reflux in ethylenediamine for 6 days led to **13** in good yields. This long reaction process can be realized in a much quicker (only 9 h) and more efficient way by use of the microwave[11] (see Experimental Section). Next, **13** was converted to

Scheme 3[a]



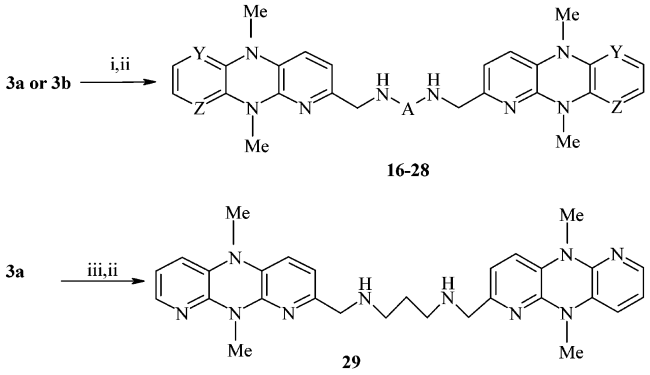
[a] (i) (1) H_2N-$(CH_2)_3$-CN,CH_2Cl_2; (2) $NaBH_4$, MeOH. (ii) H_2N-$(CH_2)_2$-NH_2, EtOH. (iii) MeOCO-$(CH_2)_2$-NMe_2-$AlMe_3$, toluene. (iv) H_2N-$(CH_2)_3$-NMe_2, MnO_2, NaCH, *i*-PrOH.

14 via a described transfunctionalization.[12] Compound **15** was prepared in one step from aldehyde **3b** by a useful amino oxidation[13] with 3-(dimethylamino)propylamine in the presence of manganese oxyde and sodium cyanide.

Cytotoxicity. The cytotoxic potential of the different monomers was evaluated in the L1210 murine leukemia cell line (Table 1), and as expected, much lower IC_{50} values were frequently (but not always) measured with the aminoalkyl derivatives compared to the unsubstituted counterparts **2** and **3**. For example, the cytotoxicity of **3b** was enhanced by a factor of about 130 when a (dimethylamino)alkylaminomethyl side chain was incorporated (compounds **7** and **8**). Methylation of the secondary amino group of the side chain of **7** as well as its incorporation into an amido group led to the clearly less cytotoxic compounds **11** and **14**. By contrast, the carboxamido derivative **15** was found to be 12 times more cytotoxic than **3b** toward the L1210 leukemia cells but 10 times less active than **7**. The potent cytotoxicities of **7** and **8** prompted us to synthesize bisdihydrodipyridopyrazines having aminoalkyl linkers. The synthesis of compounds **16**−**30** is depicted in Scheme 4 and their cytotoxic potential is given by the IC_{50} values collected in Table 2. All dimers were easily obtained in good to very good yields (see Table 2) from aldehydes **3** and the appropriate amines by the reductive amination procedure described above. Compound **23** bearing a propylaminopropyl linker was found to be the most cytotoxic compound in this "dimer" series but was in the same range of cytotoxicity as the monomers **7** and **8**.

Compounds **19** and **20** were also evaluated in vivo on the P388 leukemia. On day 0, 10^6 leukemic cells were grafted in the peritoneal cavity of B6D2F1 mice as described.[14] Administered i.v. on day 1, **20** proved to be moderately but significantly active, inducing an increase in survival of 48%, whereas **19** was devoid of antitumor activity.

Scheme 4[a]

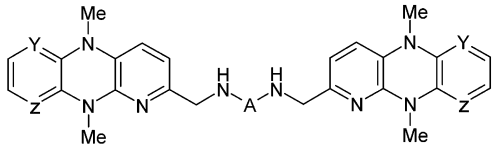


[a] (i) H_2N-A-NH_2 (A given in Table 2), CH_2Cl_2; (ii) $NaBH_4$, MeOH; (iii) **10**, CH_2Cl_2.

DNA Binding. Among the most cytotoxic compounds, seven representative dihydrodipyridopyrazines, two monomers (**7** and **8**) and five dimers (**20**, **22**, **23**, **25**, and **26**), were selected for an investigation of their DNA binding capacities. Three complementary technical approaches were deployed: melting temperature (T_m) measurements, electric linear dichroism (ELD), and DNase I footprinting, to evaluate their relative affinity for DNA, mode of binding, and sequence selectivity, respectively.

The results of the T_m analysis performed with CT DNA and poly(dAT)$_2$ are shown in Figure 1. Four typical melting curves are presented in the top panel, and the ΔT_m values ($\Delta T_m = T_m^{complex} - T_m^{DNA}$) are compared in the bottom panel. The compounds fall in two groups. The dimers **23**, **25**, and **26** proved to be very efficient at stabilizing duplex DNA against heat denaturation, whereas dimers **20** and **22** gave low ΔT_m values comparable to those observed with the monomers **7** and **8**. The differences between these two groups of compounds was also evident from absorption measurements with

Table 2. Synthesis and Cytotoxic Evaluation of Bisdihydrodipyridopyrazine Derivatives **16**−**29**



compd	Y	Z	A	yield[a] (%)	IC_{50} (μM)	cycle
16	N	CH	$(CH_2)_3$	66	4.0	NS[b] (toxic at 10 μM)
17	N	CH	$(CH_2)_7$	75	0.5	NS (toxic at 2 μM)
18	N	CH	$(CH_2)_3$-NMe-$(CH_2)_3$	69	3.5	NS (toxic at 10 μM)
19	CH	N	$(CH_2)_2$	92	2.7	79% G2M at 10 μM
20	CH	N	$(CH_2)_3$	95	0.43	68% G2M at 1 μM
21	CH	N	$(CH_2)_4$	90	1.7	NS (toxic at 5 μM)
22	CH	N	$(CH_2)_7$	60	0.71	NS (toxic at 5 μM)
23	CH	N	$(CH_2)_3$-NH-$(CH_2)_3$	60	0.36	65% G2M, 18% 8N at 1 μM
24	CH	N	$(CH_2)_3$-NMe-$(CH_2)_3$	69	1.6	72% G2M at 2.5 μM
25	CH	N	$(CH_2)_3$-NH-$(CH_2)_4$	70	0.65	75% G2M at 5 μM
26	CH	N	$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$	90	0.71	72% G2M at 5 μM
27	CH	N	$(CH_2)_2$-NH-$(CH_2)_2$-NH-$(CH_2)_2$	55	4.1	70% G2M at 20 μM
28	CH	N	$(CH_2)_3$-O-$(CH_2)_3$	66	0.68	78% G2M, 18% 8N at 5 μM
29[c]				75	0.48	66% G2M, 8N at 2 μM

[a] With respect to **3**. [b] NS, no specific effect. [c] See Scheme 4.

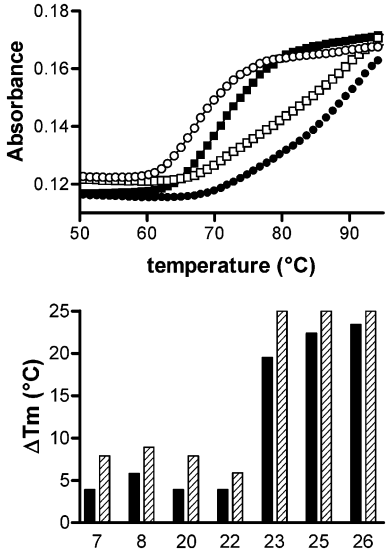


Figure 1. (a, top panel) Thermal denaturation curves for calf thymus DNA (○) in the absence and presence of (■) monomer **7** or the dimers (□) **25** and (●) **26** (at a drug−DNA ratio of 0.1). (b, bottom panel) Variation of the ΔT_m ($T_m^{drug-DNAcomplex} - T_m^{DNAalone}$, in degrees Celsius) of the complexes between the test compounds and (black bars) calf thymus DNA or (hatched bars) poly(dAT)$_2$. Melting temperature measurements were performed in BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 1 mM EDTA) at pH 7.0 with a drug/DNA ratio of 0.5.

large hypochromic and bathochromic shifts seen only with **23**, **25**, and **26** upon titration with CT DNA (data not shown).

The results of the electrooptical analysis, shown in Figure 2, attest that these compounds all behave as typical DNA-intercalating agents, with negative reduced dichroism signals observed in the characteristic drug absorption band around 350−410 nm. This technique cannot distinguish mono- and bisintercalators but easily discriminates between intercalators ($\Delta A/A < 0$) and minor-groove binders ($\Delta A/A > 0$).[15] The negative $\Delta A/A$ values recorded with the monomers and dimers upon binding to calf thymus DNA (Figure 2a,b), as well as to poly(dAT)$_2$ and poly(dGC)$_2$ (Figure 2c), indicate that they intercalate into DNA, as anticipated. Although the bisintercalation process has not been fully evidenced,

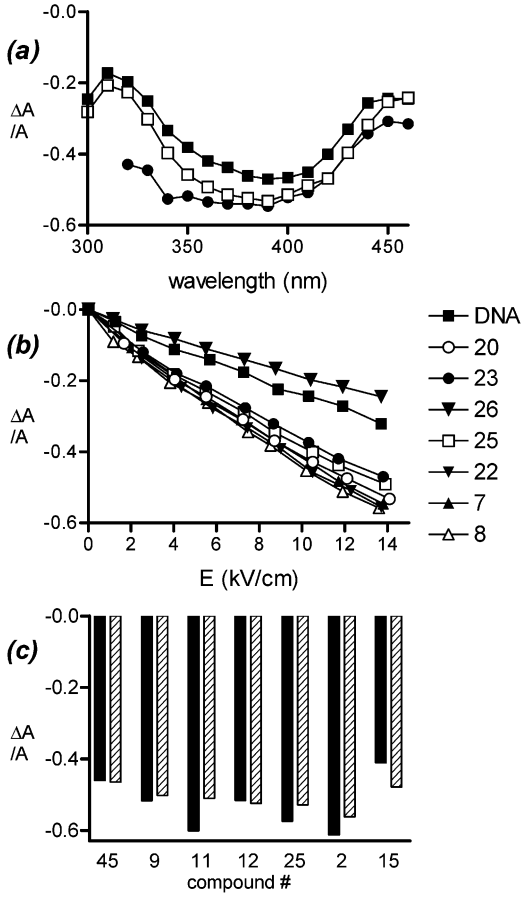


Figure 2. ELD data for the binding to DNA. Dependence of the reduced dichroism $\Delta A/A$ on (a) the wavelength and (b) electric field strength for (●) monomer **7** and dimers (□) **20** and (■) **23**. Panel c shows the variation of the reduced dichroism ($\Delta A/A$) of the complexes between the test compounds and (solid bars) poly(dAT)$_2$ and (hatched bars) poly(dGC)$_2$. Conditions: (a) 13.6 kV/cm, P/D = 20 (200 μM DNA, 10 μM drug); (b and c) 390 nm, P/D = 20 for the DNA−drug complexes and 260 nm for the DNA alone. All measurements were performed in 1 mM sodium cacodylate buffer, pH 7.0.

the fact that dimers **23**, **25**, and **26** give high T_m values (well superior compared to those obtained with monomers **7** and **8**) and efficiently unwind supercoiled DNA

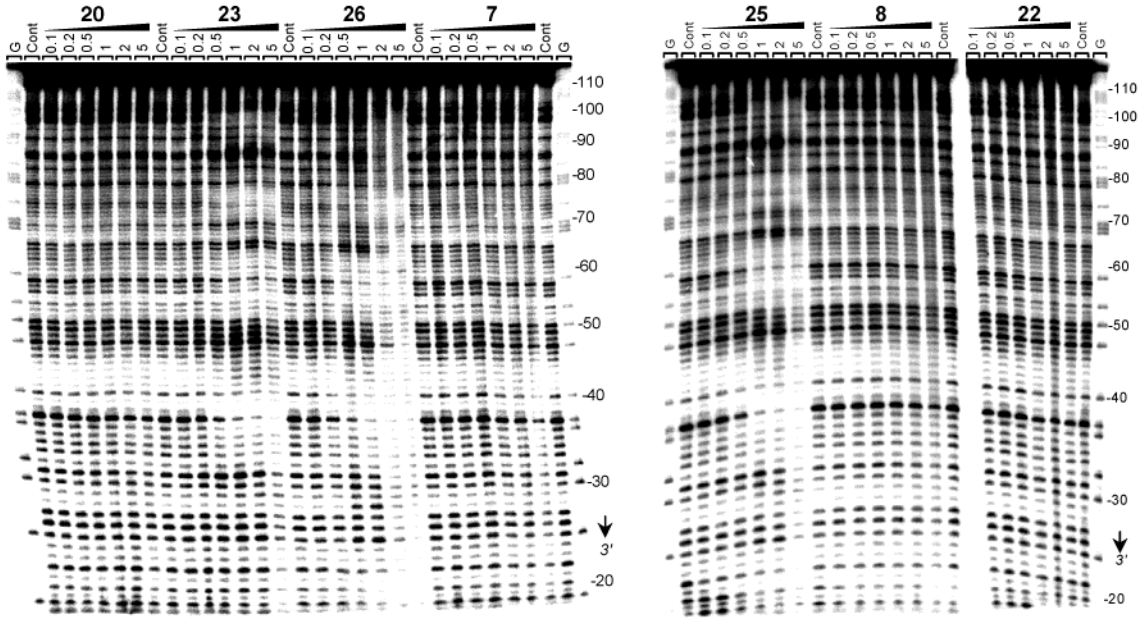


Figure 3. Sequence selective binding. DNase I footprinting with a 117-bp *Pvu*II−*Eco*RI restriction fragment in the presence of graded concentrations of the drugs. The DNA was 3′-end labeled at the *Eco*RI site with [α-^{32}P]dATP in the presence of AMV reverse transcriptase. The products of nuclease digestion were resolved on an 8% polyacrylamide gel containing 7 M urea. Control tracks (Cont) contained no drug. Guanine-specific sequence markers obtained by treatment of the DNA with dimethyl sulfate followed by piperidine were run in the lanes marked G. Numbers on the left side of the gel refer to the standard numbering scheme for the nucleotide sequence of the DNA fragment, as indicated in Figure 2.

(see below) provides a strong indication to suggest that they effectively bisintercalate.

A 117-bp ^{32}P-radiolabeled DNA fragment was used for the DNase I footprinting experiments. The drug−DNA complexes were subjected to limited cleavage by the endonuclease and the resulting fragments were resolved on polyacrylamide gels, such as those shown in Figure 3. Monomers **7** and **8** and dimers **20** and **22** showed practically no effect on the DNase I cutting profile. In contrast, the nuclease activity was considerably modified in the presence of dimers **23**, **25**, and **26**, producing clear footprints, i.e., regions where the cleavage of DNA by the enzyme is reduced adjacent to regions where the cleavage is enhanced. Densitometric analysis of the footprinting gels led us to construct differential cleavage plots from which the position and sequence of the footprint, presumptive drug binding sites, can be identified (Figure 4). The three dimers **23**, **25**, and **26**, exhibit an identical sequence selectivity with a preference for GC-rich sequences (but not necessarily pure GC tracts). Binding to runs of nonalternating AT base pairs is clearly disfavored with these compounds. In fact, their sequence recognition is reminiscent of that observed with certain bisnaphthalimides.[16]

Effect on Topoisomerases. Supercoiled plasmid DNA was used to determine whether the compounds can stimulate strand breaks by human topoisomerases I or II, but all seven monomers and dimers tested were found to be inactive in the relaxation assays. No occurrence of single-strand (topoisomerase I) or double-strand (topoisomerase II) breaks was observed, in contrast to the effect of the references camptothecin and etoposide (data not shown). The dihydrodipyridopyrazines do not stabilize topoisomerase−DNA covalent complexes. The only effect observed is nonspecific and reflects the intercalative process typified by an unwinding of the DNA double helix. As shown in Figure 5, the drugs alter the electrophoretic mobility of the topoisomers. The effect is weak with the monomers **7** and **8** but pronounced with the dimers **23**, **25**, and **26** (compare the 2.5 μM lanes). The results agree with the T_m measurements and suggest that these dimers bisintercalate in DNA.

Conclusion

In conclusion, we have identified a novel class of DNA-targeted cytotoxic agents. Dihydrodipyridopyrazine monomers and dimers form intercalation complexes with DNA and some of them exhibit a preference for GC-rich sequences. DNA can be considered as a bioreceptor for these compounds, but the protein partners are as yet unknown. While the precise mode of action of these compounds is not yet understood, their potent cytotoxic properties is a good augur for the development of more active analogues.

Experimental Section

Chemistry: General Methods. Mps were determined on a Tottoli melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with a Bruker AM 400 or a Bruker 250 MHz spectrometer (Attached Proton Test method, APT) or a Bruker instrument Avance DPX250 at 250.131 and 62.9 MHz, respectively. Chemical shifts (δ values) were reported in parts per million and coupling constants (J values) in Hz. Me_4Si was the internal standard. Infrared spectra were recorded using NaCl film or KBr pellets techniques on a Perkin-Elmer spectrometer FT PARAGON 1000PC or on a Perkin-Elmer 841 instrument. Elemental analyses were performed by CNRS laboratory (Vernaison). Mass spectra (MS) were recorded on a Perkin-Elmer mass spectrometer SCIEX API 300 by ion spray (IS). The electronic impact high-resolution mass measurements were obtained on a Finnigan MAT 95 Q mass spectrometer. TLC were performed with plates coated with Kieselgel G (Merck). The silica gel used for flash chromatography was Kieselgel of 0.04−0.063 mm particle size. The eluent used was either Et_3N/MeOH 1:1 (eluent A) or CH_2Cl_2/MeOH/NH_4OH 30% 100/10/1 (eluent B).

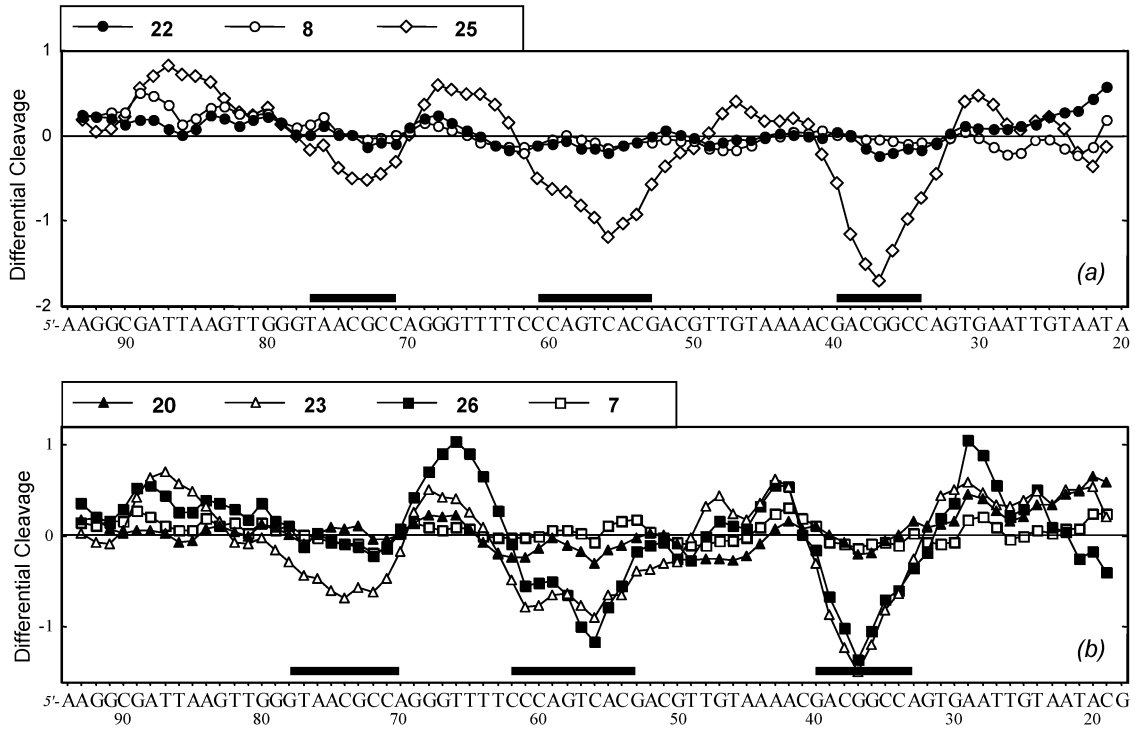


Figure 4. Differential cleavage plots comparing the susceptibility of the 117-bp DNA fragment to DNase I cutting in the presence of the monomers and dimers (1 μM each). Deviation of points toward the lettered sequence (negative values) corresponds to a ligand-protected site, and deviation away (positive values) represents enhanced cleavage. Vertical scales are in units of ln (f_a) − ln (f_c), where f_a is the fractional cleavage at any bond in the presence of the drug and f_c is the fractional cleavage of the same bond in the control, given a closely similar extent of digestion in each case.

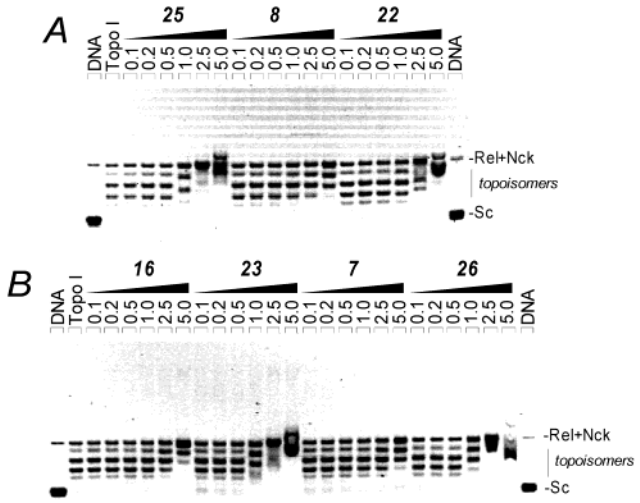


Figure 5. Effect of the drugs on the relaxation of plasmid DNA by human topoisomerases I. Native supercoiled pKMp27 DNA (0.5 μg) (lane DNA) was incubated with 4 units of topoisomerase in the absence (lane Topo) or presence of graded concentrations (given as micromolar) of the indicated compound. Reactions were stopped with sodium dodecyl sulfate and treatment with proteinase K. DNA samples were separated by electrophoresis on agarose gels and the gels were stained with ethidium bromide (1 μg/mL) prior to photography under UV light. Nck, nicked; Rel, relaxed; Sc, supercoiled.

Where analyses are indicated by the symbols of the elements, analytical results obtained for those elements were ±0.3% of the theoretical values.

Sodium amide powder was obtained commercially (Merck). Reagent-grade tetrahydrofuran (THF) was first distilled from potassium hydroxide and then from sodium benzophenone ketyl and stored over sodium until used.

Synthewave S402 Prolabo microwave reactor (monomode system, 2450 MHz, 300 W) which has variable-speed rotation, visual control, irradiation monitored by PC computer, IR measurement and continuous feedback temperature control by PC.

The Boc-propylene diamine was synthezised by reaction of the 1,3-diaminopropane with di-*tert*-butyl dicarbonate in chloroform.[17] 4-(Dimethylamino) butylamine and 5-(dimethylamino)pentylamine were obtained from 4-bromobutyronitrile and 5-valeronitrile respectively according a procedure reported in the literature.[18] All other amino precursors were commercially available. Compounds **2a,b** and **3a,b** were prepared according to ref 7.

Reductive Amination of Aldehydes 3: Preparation of Compounds 4−9. A typical procedure is exemplified by the preparation of the compound **4** (Table 1). The reaction was performed under argon atmosphere.To a 100 mL flask was added a magnetic stir bar and a solution of 2 mmol of **3a** in 25 mL of methylene chloride. A solution of 7.2 mmol of *N*,*N*-dimethylethylenediamine in 31 mL of methylene chloride was then rapidly added. After 1 h at room temperature (the reaction was monitored by NMR), the solvent was removed under vacuum and replaced by 25 mL of methanol. After completion the reaction mixture was added to ice−water, extracted with methylene chloride and the organic layer dried over $MgSO_4$. After removal of the solvent under vacuum, the residue was subjected to flash chromatography on silica gel (eluent A) to afford **4**.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:2,3-*e*]-pyrazin-2-yl)methyl]-N2(dimethyl)-1,2-ethanediamine 4:** gum, IR (NaCl) 3312 cm^{-1} (NH). ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 6H), 2.43 (t, J = 6 Hz, 2H), 2.56 (br s, 1H), 2.68 (t, J = 6 Hz, 2H), 3.03 (s, 3H), 3.06 (s, 3H), 3.56 (s, 2H), 6.27−6.42 (m, 4H), 7.38 (d, J = 5 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 28.33, 28.58, 45.33, 46.30, 53.87, 58.97, 114.26, 114.64, 115.34, 116.22, 130.27, 131.88, 138.01, 146.98, 147.51, 147.88. Anal. $C_{17}H_{24}N_6$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:2,3-*e*]-pyrazin-2-yl)methyl]-*N*3(dimethyl)-1,3-propanediamine 5:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3418 cm^{-1} (NH). ^{1}H NMR ($CDCl_3$) δ 1.69 (q, J = 7.3 Hz, 2H), 2.21 (s, 6H), 2.33 (t, J = 7.3 Hz, 2H), 2.65

(t, J = 6.9 Hz, 2H), 2.82 (br s, 1H), 3.02 (s, 3H), 3.04 (s, 3H), 3.53 (s, 2H), 6.27 (d, J = 7.6 Hz, 1H), 6.31 (dd, J = 1.3 and 7.6 Hz, 1H), 6.37 (d, J = 7.6 Hz, 1H), 6,40 (dd, J = 4.9 and 7.6 Hz, 1H), 7.43 (dd, J = 1.3 and 4.9 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 27.7, 28.6, 28.8, 45.4, 47.5, 53.8, 58.0 114.5, 115.1, 115.5, 116.4,130.6, 132.0, 138.3, 147.3, 147.5, 147.7. HRMS calcd for $C_{18}H_{26}N_6$ $[M + H]^+$ 327.2297, found 327.2292. Anal. $C_{18}H_{26}N_6$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]-pyrazin-2-yl)methyl]-*N*2-(dimethyl)-1,2-ethanediamine 6:** obtained as a gum after column chromatography (eluent A), IR (NaCl) 3311 cm^{-1} (NH). ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 6H), 2.31 (m, 1H), 2.44 (t, J = 6 Hz, 2H), 2.69 (t, J = 6 Hz, 2H), 2.82 (s, 3H), 3.29 (s, 3H), 3.59 (s, 2H), 6.24−6.49 (m, 4H), 7.46 (d, J = 4.5 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 27.60, 30.80, 45.37, 46.39, 53.93, 59.06, 114.09, 114.64, 114.70, 116.75, 130.32, 132.06, 137,89, 147.97, 148.04, 148.59. Anal. $C_{17}H_{24}N_6$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]-pyrazin-2-yl)methyl]-*N*3-(dimethyl)-1,3-propanediamine 7:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3416 cm^{-1} (NH). ^{1}H NMR ($CDCl_3$) δ 1.71 (qt, J = 6.9 Hz, 2H), 2.22 (s, 6H), 2.34 (t, J = 6.9 Hz, 2H), 2.68 (t, J = 6.9 Hz, 2H), 2.79 (s, 3H), 2.84 (br s, 1H), 3.27 (s, 3H), 3.57 (s, 2H), 6.23 (d, J = 7.9 Hz, 1H), 6.26 (dd, J = 1.2 and 7.4 Hz, 1H), 6.41 (d, J = 7.9 Hz, 1H), 6.46 (dd, J = 5.2 and 7.4 Hz, 1H), 7.43 (dd, J = 1.2 and 5.2 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 27.6, 27.7, 30.8, 45.4, 47.6, 53.8, 58.0, 114.2, 114.7, 116.8, 130.4, 132.1, 137.9, 147.5, 148.1, 148.6. Anal. $C_{18}H_{26}N_6$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]-pyrazin-2-yl)methyl]-*N*4-(dimethyl)-1,4-butanediamine 8:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3430 cm^{-1} (NH). ^{1}H NMR ($CDCl_3$) δ 1.52−1.55 (m, 4H), 2.20 (s, 6H), 2.27 (t, J = 7.0 Hz, 2H), 2.64 (t, J = 7.0 Hz, 2H), 2.83 (s, 3H), 2.85 (br s, 1H), 3.28 (s, 3H), 3.57 (s, 2H), 6.24 (d, J = 7.6 Hz, 1H), 6.27 (dd, J = 1.2 and 7.6 Hz, 1H), 6.43 (d, J = 7.6 Hz, 1H), 6.46 (dd, J = 4.9 and 7.6 Hz, 1H), 7.44 (dd, J = 1.2 and 4.9 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 25.4, 27.7, 27.9, 30.9, 45.3, 49.1, 53.9, 59.6, 114.2, 114.7, 114.8, 116.8, 130.5, 132.1, 137.9, 147.9, 148.2, 148.7. Anal. $C_{19}H_{28}N_6$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]-pyrazin-2-yl)methyl]-*N*5-(dimethyl)-1,5-pentanediamine 9:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3438 cm^{-1} (NH). ^{1}H NMR ($CDCl_3$) δ 1.34−1.60 (m, 6H), 2.20 (s, 6H), 2.25 (t, J = 7.0 Hz, 2H), 2.35 (br s, 1H), 2.61 (t, J = 7.0 Hz, 2H), 2.80 (s, 3H), 3.27 (s, 3H), 3.56 (s, 2H), 6.20−6.27 (m, 2H), 6.41 (d, J = 7.6 Hz, 1H), 6.46 (dd, J = 5.2 and 7.6 Hz, 1H), 7.44 (dd, J = 1.2 and 5.2 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 25.1, 27.5, 27.8, 27.8, 31.0, 45.4, 49.1, 53.9, 59.6, 114.3, 114.8, 114.9, 116.9, 130.6, 132.2, 138.1, 147.7, 148.3, 148.8. Anal. $C_{20}H_{30}N_6$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]-1,3-propanediamine 10.** The *tert*-butoxycarbonyl intermediate (Scheme 2) was obtained from the corresponding monoprotected propylenediamine according to the reductive amination process described above. The compound was obtained as a gum in 98% yield after column chromatography (eluent B).

Then 0.41 mmol of monoprotected product **10** was dissolved in 2 mL of formic acid. After 4 h of stirring at room temperature, the reaction medium was concentrated under vacuum and then diluted with methylene chloride. The organic layer was washed with a saturated solution of Na_2CO_3 and dried over $MgSO_4$. **10** was obtained as a gum after column chromatography (eluent B). IR (NaCl) 3416 cm^{-1} (NH). ^{1}H NMR ($CDCl_3$) δ 1.67 (qt, J = 6.9 Hz, 2H), 1.82 (br s, 3H), 2.67 (t, J = 6.9 Hz, 2H), 2.78 (t, J = 6.9 Hz, 2H), 2.82 (s, 3H), 3.28 (s, 3H), 3.56 (s, 2H), 6.25 (d, J = 7.8 Hz, 1H), 6.28 (dd, J = 1.5 and 7.8 Hz, 1H), 6.42 (d, J = 7.8 Hz, 1H), 6.48 (dd, J = 5.0 and 7.8 Hz, 1H), 7.45 (dd, J = 1.5 and 5.0 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 27.7, 31.0, 33.8, 40.4, 46.9, 54.0, 114.3, 114.8, 114.9, 116.9, 130.6, 132.2, 138.1, 147.9, 148.3, 148.7. HRMS calcd for $C_{33}H_{42}N_{11}$ $[M + H]^+$ 299.1984, found 299.1986. Anal. $C_{16}H_{22}N_6$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]-pyrazin-2-yl)methyl]-*N*3-(dimethyl)-*N*1-(methyl)-1,3-propanediamine 11.** To a solution of 0.31 mL of **7** in 2 mL of methanol was added 0.25 mL of aqueous formaldehyde (37%). After the mixture was stirred for 6 h at room temperature, 3.1 mmol of $NaBH_4$ was added at 0 °C; the reaction mixture was stirred for 2 h at room temperature and then hydrolyzed at 0 °C and extracted with methylene chloride. The organic layer was dried over $MgSO_4$. Compound **11** was obtained as a gum after column chromatography (eluent B). IR (NaCl) 3416 cm^{-1} (NH). ^{1}H NMR ($CDCl_3$) δ 1.71 (qt, J = 7.6 Hz, 2H), 2.23 (s, 6H), 2.27 (s, 3H), 2.30 (t, J = 7.6 Hz, 2H), 2.44 (t, J = 7.6 Hz, 2H), 2.81 (s, 3H), 3.28 (s, 3H), 3.38 (s, 2H), 6.26 (dd, J = 1.2 and 7.6 Hz, 2H), 6.28 (d, J = 7.9 Hz, 2H), 6.45 (dd, J = 5.2 and 7.6 Hz, 2H), 6.52 (d, J = 7.9 Hz, 2H), 7.43 (dd, J = 1.2 and 5.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 25.6, 27.7, 30.9, 42.5, 45.5, 55.3, 57.3, 62.3, 114.1, 115.0, 115.9, 116.8, 130.3, 132.2, 138.1, 147.4, 147.7, 148.9. Anal. $C_{19}H_{28}N_6$.

***N*2-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]-pyrazin-2-yl)methyl]-3-aminopropionitrile 12.** To a solution of 0.41 mmol of aldehyde **3b** in 5 mL of methylene chloride was added under argon atmosphere 1.23 mmol of 3-aminopropionitrile. After stirring for 1 h at room temperature, the solution was concentrated under vacuum. The residue was dissolved in 5 mL of MeOH, and 4.1 mmol of $NaBH_4$ was added. After stirring for 1 h at room temperature, the reaction medium was hydrolyzed at 0 °C and extracted with methylene chloride. The organic layer was dried over $MgSO_4$. The compound **12** was obtained as a gum in 87% yield after column chromatography (eluent B). IR (NaCl) 2364 cm^{-1} (CN). ^{1}H NMR ($CDCl_3$) δ 1.94 (br s, 1H), 2.54 (t, J = 6.7 Hz, 2H), 2.83 (s, 3H), 2.94 (t, J = 6.7 Hz, 2H), 3.28 (s, 3H), 3.61 (s, 2H), 6.26 (d, J = 7.6 Hz, 1H), 6.30 (dd, J = 1.2 and 7.6 Hz, 1H), 6.42 (d, J = 7.6 Hz, 1H), 6.48 (dd, J = 5.2 and 7.6 Hz, 1H), 7.46 (dd, J = 1.2 and 5.2 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 18.6, 27.6, 30.8, 44.3, 53.1, 114.3, 114.6, 114.7, 116.9, 118.7, 130.7, 132.0, 138.0, 146.9, 148.3, 148.5. Anal. $C_{16}H_{18}N_6$.

5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methylamine 13. To a solution of 0.85 mmol of the nitrile **12** in 10 mL of ethanol was added 51 mmol of ethylenediamine. The mixture was refluxed for 6 days or submitted to microwaves (P = 20%, 100 °C) for 9 h. The mixture was concentrated under vacuum. The compound **13** was obtained as a gum in 72% yield after chromatography (eluent B). IR (NaCl) 3260 cm^{-1} (NH_2). ^{1}H NMR ($CDCl_3$) δ 1.98 (br s, 1H), 2.55 (t, J = 6.7 Hz, 2H), 2.82 (s, 3H), 2.90 (t, J = 6.7 Hz, 2H), 3.29 (s, 3H), 3.58 (s, 2H), 3.68 (s, 3H), 6.25 (d, J = 7.6 Hz, 1H), 6.29 (dd, J = 1.2 and 7.6 Hz, 1H), 6.41 (d, J = 7.6 Hz, 1H), 6.47 (dd, J = 5.2 and 7.6 Hz, 1H), 7.45 (dd, J = 1.2 and 5.2 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 27.8, 31.1,34.6, 44.4, 51.6, 53.8, 114.4, 114.8, 114.9, 117.0, 130.9, 132.2, 138.2, 146.9, 148.5, 162.1. Anal. $C_{13}H_{15}N_5$.

3-(Dimethylamino)-*N*-[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]propanamide 14. To a solution of 0.40 mmol of amine **13** in 2 mL of toluene was added under argon atmosphere 0.40 mmol of $AlMe_3$ in solution in toluene (2 mol/L). After the mixture was stirred for 1 h at room temperature, 0.40 mmol of methyl 3-aminopropionate was added and the mixture was refluxed for 12 h. After hydrolysis, Celite filtration, and extraction with methylene chloride, the organic layer was dried over $MgSO_4$. The compound **14** was obtained as a gum after column chromatography (eluent B). IR (NaCl) 3418 (NH), 1651 cm^{-1} (CO). ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 6H), 2.42 (t, J = 5.9 Hz, 2H), 2.58 (t, J = 5.9 Hz, 2H), 2.82 (s, 3H), 3.29 (s, 3H), 4.25 (d, J = 5.2 Hz, 2H), 6.26 (d, J = 7.6 Hz, 1H), 6.27−6.31 (m, 1H), 6.42 (d, J = 7.6 Hz, 1H), 6.48 (dd, J = 5.2 and 7.6 Hz, 1H), 7.45 (dd, J = 1.2 and 5.2 Hz, 1H), 8.54 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 26.9, 27.7, 31.2, 38.5, 45.5, 58.4, 114.3, 115.4, 117.2, 117.3, 131.3, 134.7, 138.9, 139.0, 147.0, 148.1, 164.5. Anal. $C_{18}H_{24}N_6O$.

***N*-[3-(Dimethylamino)propyl]-5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-carboxamide 15.** To a solution of 4 mL of 3-dimethylaminopropylamine in 4 mL of 2-propanol was added under argon atmosphere 2.08 mmol of NaCN. After the mixture was stirred for 5 min at room temperature, 0.42 mmol of aldehyde **3b** and 4.2 mM of freshly

prepared[19] MnO_2 were added at 0 °C. After 10 min, 4.2 mmol of MnO_2 was again added; the mixture was stirred for 4 h and then filtered over Celite, which was washed several times with methanol. The compound **15** was obtained as a gum after column chromatography (eluent B). IR (NaCl) 3442 (NH), 1682 cm^{-1} (CO). ^1H NMR ($CDCl_3$) δ 1.74 (qt, J = 6.7 Hz, 2H), 2.26 (s, 6H), 2.44 (t, J = 6.7 Hz, 2H), 2.87 (s, 3H), 3.29 (s, 3H), 3.50 (m, 2H), 6.33 (d, J = 7.9 Hz, 1H), 6.36 (dd, J = 1.2 and 7.9 Hz, 1H), 6.51 (dd, J = 5.2 and 7.9 Hz, 1H), 7.39 (d, J = 7.9 Hz, 1H), 7.49 (dd, J = 1.2 and 5.2 Hz, 1H), 8.14 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 26.9, 27.7, 31.2, 38.5, 45.5, 58,4, 114,3, 115,4, 117,2, 117,3, 131,3, 134,7, 138.9, 139.0, 147.0, 148.1, 164.5. Anal. $C_{18}H_{24}N_6O$.

Preparation of Compounds 16−29. The same procedure was used for the preparation of compounds **16−29** (Table 2) except that the diamines were added as 0.5 equiv with respect to **3**.

***N*1,*N*3-Di[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:2,3-*e*]pyrazin-2-yl)methyl]-1,3-propanediamine 16:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3500−3300 cm^{-1}(NH). ^1H NMR ($CDCl_3$) δ 1.79 (qt, J = 6.6 Hz, 2H), 2.39 (br s, 2H), 2.75 (t, J = 6.6 Hz, 4H), 3.02 (s, 6H), 3.03 (s, 6H), 3.56 (s, 4H), 6.27 (d, J = 7.6 Hz, 2H), 6.31 (dd, J = 1.4 and Hz, 2H), 6.37 (d, J = 7.6 Hz, 2H), 6.41 (dd, J = 5.2 and 7.6 Hz, 2H), 7.38 (dd, J = 1.4 and 5.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 27.7, 29.3, 31.4, 48.3, 54.1, 114.7, 115.3, 115.4, 117.4, 130.6, 132.1, 138.5, 147.2, 147.8, 148.6. Anal.$C_{29}H_{34}N_{10}$.

***N*1,*N*3-Di[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:2,3-*e*]pyrazin-2-yl)methyl]-1,7-heptanediamine 17:** obtained as a gum after chromatography (eluent B), IR (NaCl) 3418 cm^{-1} (NH). ^1H NMR ($CDCl_3$) δ 1.28−1.36 (m, 6H), 1.49−1.52 (m, 4H), 2.03 (br s, 2H), 2.59 (t, J = 6.9 Hz, 4H), 3.05 (s, 6H), 3.06 (s, 6H), 3.54 (s, 4H), 6.29 (d, J = 7.8 Hz, 2H), 6.33 (dd, J = 1.3 and 7.5 Hz, 2H), 6.38 (d, J = 7.8 Hz, 2H), 6.42 (dd, J = 5.0 and 7.5 Hz, 2H), 7.38 (dd, J = 1.3 and 5.0 Hz, 2H). ^{13}C NMR δ 27.3, 28.6, 28.8, 29.4, 29.9, 49.2, 53.9, 114.5, 115.1, 115.6, 116.4, 130.6, 132.1, 138.3, 147.3, 147.8, 147.9. HRMS calcd for $C_{33}H_{42}N_{10}$ $[M + H]^+$ 579.3672, found 579.3674. Anal.$C_{33}H_{42}N_{10}$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:2,3-*e*]pyrazin-2-yl)methyl]-*N*3-(3-{[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:2,3-*e*]pyrazin-2-yl)methyl]amino}-*N*3-methyl-1,3-propanediamine 18:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3418 cm^{-1}(NH). ^1H NMR ($CDCl_3$) δ 1.70 (q, J = 6.9 Hz, 4H), 2.09 (br s, 2H), 2.22 (s, 3H), 2.40 (t, J = 6.9 Hz, 4H), 2.64 (t, J = 6.9 Hz, 4H), 3.03 (s, 6H), 3.05 (s, 6H), 3.52 (s, 4H), 6.27 (d, J = 7.5 Hz, 2H), 6.32 (dd, J = 1.6 and 7.5 Hz, 2H), 6.37 (d, J = 7.5 Hz, 2H), 6.42 (dd, J = 5.0 and 7.5 Hz, 2H), 7.38 (dd, J = 1.6 and 5.0 Hz, 2H). HRMS calcd for $C_{33}H_{42}N_{11}$ $[M + H]^+$ 594.3781, found 594.3779. Anal.$C_{33}H_{43}N_{11}$.

***N*1,*N*2-Di[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]-1,2-ethylenediamine 19:** obtained as a solid (mp = 171 °C) after column chromatography (eluent B), IR (NaCl) 3279 cm^{-1}(NH). ^1H NMR ($CDCl_3$) δ 2.48 (m, 2H), 2.78 (s, 6H), 2.79 (s, 4H), 3.24 (s, 6H), 3.57 (s, 4H), 6.23 (m, 4H), 6.43 (m, 4H), 7.42 (d, J = 4.5 Hz, 2H). ^{13}C NMR δ 27.5, 30.6, 48.3, 53.5, 113.9, 114.5, 114.5, 116.6, 130.2, 131.8, 137.6, 147.5, 147.8, 148.2. Anal.$C_{28}H_{32}N_{10}$.

***N*1,*N*3-Di[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]-1,3-propanediamine 20:** obtained as a solid (mp = 61 °C) after column chromatography (eluent B), IR (NaCl) 3560−3260 cm^{-1}(NH). ^1H NMR ($CDCl_3$) δ 1.85 (qt, J = 6.7 Hz, 2H), 2.76 (s, 6H), 2.83 (t, J = 6.7 Hz, 4H), 3.21 (s, 6H), 3.61 (s, 4H), 6.21 (d, J = 7.6 Hz, 2H), 6.24 (dd, J = 1.2 and 7.6 Hz, 2H), 6.43 (d, J = 7.6 Hz, 2H), 6.46 (dd, J = 5.2 and 7.6 Hz, 2H), 7.43 (dd, J = 1.2 and 5.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 27.7, 29.2, 30.9, 47.8, 53.7, 114.2,-114.8, 115.0, 116.9, 130.6, 132.1, 138.0, 147.2, 148.2, 148.7. HRMS calcd for $C_{29}H_{34}N_{10}$ $[M + H]^+$ 522.2962, found 522.2980. Anal.$C_{29}H_{34}N_{10}$.

***N*1,*N*4-Di[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]-1,4-butanediamine 21:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3500−3200 cm^{-1}(NH). ^1H NMR ($CDCl_3$) δ 1.63 (m, 4H), 2.67 (m, 4H), 2.78 (s, 6H), 3.25 (s, 6H), 3.57 (s, 4H), 6.21−6.26 (m, 4H), 6.41−6.47 (m, 4H), 7.44 (d, J = 4.5 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 27.7, 27.9, 30.9, 48.9, 53.6, 114.3, 114.8, 114.9, 116.9, 130.6, 132.1, 138.0, 147.2, 148.2, 148.6. Anal.$C_{30}H_{36}N_{10}$.

***N*1,*N*7-Di[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]-1,7-heptanediamine 22:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3406 cm^{-1}(NH). ^1H NMR ($CDCl_3$) δ 1.32 (m, 6H), 1.44−1.58 (m, 4H), 2.22 (s, 2H), 2.59 (t, J = 7.1 Hz, 4H), 2.79 (s, 6H), 3.27 (s, 6H), 3.55 (s, 4H), 6.23 (d, J = 7.8 Hz, 2H), 6.25 (dd, J = 1.2 and 5.0 Hz, 2H), 6.41 (d, J = 8.4 Hz, 2H), 6.44 (dd, J = 5.0 and 7.8 Hz, 2H), 7.43 (dd, J = 1.2 and 5.0 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 27.7, 28.2, 29.3, 29.8, 30.9, 49.1, 53.8, 114.2, 114.8, 116.9, 130.5, 132.2, 138.0, 147.7, 148.2, 148.7. Anal.$C_{33}H_{42}N_{10}$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]-*N*3-(3-{[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]amino}-propyl)-1,3-propanediamine 23:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3423 cm^{-1}(NH). ^1H NMR ($CDCl_3$) δ 1.76 (q, J = 6 Hz, 4H), 2.70 (t, J = 6.6 Hz, 4H), 2.76 (t, J = 6.6 Hz, 4H), 2.78 (s, 6H), 3.25 (s, 6H), 3.54 (s, 4H), 6.22 (d, J = 7.6 Hz, 2H), 6.25 (dd, J = 1.3 and 8.0 Hz, 2H), 6.41 (d, J = 7.6 Hz, 2H), 6.45 (dd, J = 5.0 and 8.0 Hz, 2H), 7.43 (dd, J = 1.3 and 5.0 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 27.7, 28.6, 30.9, 47.6, 48.4, 53.6, 114.3, 114.9, 117.0, 130.6, 132.2, 138.0, 147.4, 148.2, 148.6. Anal. $C_{33}H_{41}N_{11}$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]-*N*3-(3-{[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]amino}-propyl)-*N*3-methyl-1,3-propanediamine 24:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3396 cm^{-1} (NH). ^1H NMR ($CDCl_3$) δ 1.69 (q, J = 6.8 Hz, 4H), 2.21 (s, 3H), 2.37−2.43 (m, 4H), 2.65 (t, J = 7.0 Hz, 4H), 2.79 (s, 6H), 3.26 (s, 6H), 3.55 (s, 4H), 6.21 (d, J = 7.5 Hz, 2H), 6.25 (dd, J = 1.3 and 5.2 Hz, 2H), 6.40 (d, J = 7.7 Hz, 2H), 6.44 (dd, J = 5.0 and 7.5 Hz, 2H), 7.42 (dd, J = 1.3 and 5.0 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 27.3, 27.7, 30.9, 42.2, 47.8, 53.9, 56.0, 114.2, 114.8, 114.8, 116.8, 130.4, 132.1, 137.9, 147.6, 148.1, 148.6. HRMS calcd for $C_{33}H_{43}N_{11}$ $[M + H]^+$ 594.3781, found 594.3798. Anal. $C_{33}H_{42}N_{10}$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]-*N*3-(3-{[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl] amino}butyl-1,3-propanediamine 25:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3430 cm^{-1}(NH). ^1H NMR ($CDCl_3$) δ 1.57 (m, 4H), 1.72 (qt, J = 6.7 Hz, 2H), 2.56 (br s, 3H), 2.60−2.74 (m, 8H), 2.78 (s, 6H), 3.26 (s, 6H), 3.54 (s, 4H), 6.20−6.26 (m, 4H), 6.41 (d, J = 7.6 Hz, 2H), 6.45 (dd, J = 5.2 and 7.6 Hz, 2H), 7.42 (dd, J = 1.2 and 5.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 27.5, 27.7, 27.8, 29.5, 30.8, 47.5, 48.1, 48.9, 49.5, 53.7, 53.8, 114.1, 114.6, 114.7, 114.8, 116.8, 130.3, 130.4, 132.1, 137.9, 147.7, 147.8, 148.0, 148.6. HRMS calcd for $C_{33}H_{43}N_{11}$ $[M + H]^+$ 594.3781, found 594.3780. Anal. $C_{33}H_{43}N_{11}$.

***N*1,*N*4-Di[{(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]amino}propyl)-1,4-butanediamine 26:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3310−3460 cm^{-1} (NH). ^1H NMR ($CDCl_3$) δ 1.50 (m, 4H), 1.70 (qt, J = 6.9 Hz, 4H), 1.79 (br s, 4H), 2.60−2.70 (m, 12H), 2.81 (s, 6H), 3.28 (s, 6H), 3.56 (s, 4H), 6.24 (d, J = 7.8 Hz, 2H), 6.27 (dd, J = 1.2 and 7.5 Hz, 2H), 6.41 (d, J = 7.8 Hz, 2H), 6.46 (dd, J = 5.2 and 7.5 Hz, 2H), 7.44 (dd, J = 1.2 and 5.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 27.7, 28.6, 30.2, 30.9, 47.6, 48.3, 49.8, 54.0, 114.3, 114.7, 114.9, 116.9, 130.5, 132.2, 138.1, 148.1, 148.2, 148.8. Anal. $C_{36}H_{50}N_{12}$.

***N*1,*N*2-Di[{(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]amino}ethyl)-1,2-ethanediamine 27:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3345 cm^{-1} (NH). ^1H NMR ($CDCl_3$) δ 2.39 (br s, 4H), 2.75 (s, 12H), 2.78 (s, 6H), 3.26 (s, 6H), 3.56 (s, 4H), 6.22 (d, J = 7.6 Hz, 2H), 6.25 (dd, J = 1.2 and 7.3 Hz, 2H), 6.42 (d, J = 7.6 Hz, 2H), 6.45 (dd, J = 5.2 and 7.3 Hz, 2H), 7.43 (dd, J = 1.2 and 5.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 27.6,

30.9, 48.5, 49.1, 49.2, 53.8, 114.2, 114.7, 114.8, 116.9, 130.5, 132.1, 138.0, 147.8, 148.2, 148.7. HRMS calcd for $C_{32}H_{42}N_{12}$ $[M + H]^+$ 595.3733, found 595.3735. Anal. $C_{32}H_{42}N_{12}$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]-pyrazin-2-yl)methyl]-*N*3-(3-{[(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]amino}-propyl)-*N*3-methyl-1,3-propanediamine 28:** obtained as a gum after column chromatography (eluent B), IR (NaCl) 3352 cm^{-1}(NH). ^1H NMR ($CDCl_3$) δ 1.72 (qt, $J = 6.0$ Hz, 4H), 2.80 (s, 6H), 2.84 (t, $J = 6.0$ Hz, 4H), 3.26 (s, 6H), 3.55 (s, 4H), 3.78 (t, $J = 6.0$ Hz, 4H), 6.23 (d, $J = 7.6$ Hz, 2H), 6.28 (dd, $J = 1.2$ and 7.6 Hz, 2H), 6.39 (d, $J = 7.6$ Hz, 2H), 6.47 (dd, $J = 5.2$ and 7.6 Hz, 2H), 7.43 (dd, $J = 1.2$ and 5.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 27.7, 30.7, 30.9, 48.4, 53.5, 63.4, 114.4, 114.8, 114.9, 116.9, 130.7, 132.1, 137.9, 147.0, 148.3, 148.6. Anal. $C_{32}H_{40}N_{10}O$.

***N*1-[(5,10-Dimethyl-5,10-dihydrodipyrido[2,3-*b*:2,3-*e*]-pyrazin-2-yl)methyl]-*N*3-([(5,10-dimethyl-5,10-dihydrodipyrido[2,3-*b*:3,2-*e*]pyrazin-2-yl)methyl]-1,3-propanediamine 29:** obtained as a gum after chromatography (eluent B), IR (NaCl) 2924 and 3500−3300 cm^{-1} (NH). ^1H NMR ($CDCl_3$) δ 1.79 (qt, $J = 6.9$ Hz, 2H), 2.49 (br s, 2H), 2.70−2.74 (m, 4H), 2.76 (s, 3H), 3.00 (s, 3H), 3.01 (s, 3H), 3.24 (s, 3H), 3.54 (s, 2H), 3.56 (s, 2H), 6.19−6.30 (m, 4H), 6.35−6.47 (m, 4H), 7.35 (dd, $J = 1.6$ and 5.0 Hz, 1H), 7.42 (dd, $J = 1.2$ and 5.0 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 27.7, 28.5, 28.7, 29.7, 30.9, 47,6, 47,7, 53,8, 114,2, 114,5, 114,8, 115,0, 115,5, 116,4, 116.9, 130.5, 132.0, 132.1, 138.0, 138.2, 147.2, 147.6, 147.7, 148.2, 148.6. Anal. $C_{29}H_{34}N_{10}$.

Cell Culture and Cytotoxicity. L1210 cells were provided by the National Cancer Institute, Frederick, MD. They were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM l-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.[20] Cells were exposed to graded concentrations of the compounds (nine serial dilution in triplicate) for 48 h. Results were expressed as IC_{50}, the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls.

For the cell cycle analysis, L1210 cells (2.5×10^5 cells/mL) were incubated for 21 h with various concentrations of the compounds. Cells were then fixed with 70% ethanol (v/v), washed, and incubated in PBS containing 100 μg/mL RNase and 25 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10^4 cells were analyzed on an ATC3000 flow cytometer (Bruker, France) with a argon laser (Spectra-Physics) emitting 400 mW at 488 nm. The fluorescence of propidium iodide was collected through a 615 nm long-pass filter.

Data are displayed as linear histograms and results are expressed as the percentage of cells found in the different phases of the cell cycle.

Antitumor Activity. The antitumor activity of the compounds was evaluated on the P388 leukemia murine model.[14] P388cells (NCI, Frederick) were inoculated *i.p.* (10^6 cells/mouse) into B6D2F1 mice (Iffa credo) on day 0. The drug were injected *i.v.* on day one. The results are expressed in terms of percent *T/C* (median survival time of treated animals divided by median survival time of controls, X 100).

Molecular Pharmacology. The experimental procedures used for the melting temperature,[21] electric linear dichroism,[15] and DNase I footprinting[22] experiments have been previously described.

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References

(1) (a) Le Pecq, J. B.; Le Bret, M.; Barbet, J.; Roques, B. DNA polyintercalating drugs: DNA binding of diacridine derivatives. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2915−2919. (b) Denny, W. A.; Baguley, B. C.; Cain, B. F.; Waring, M. J. Antitumour acridines. *Molecular aspects of anti-cancer drug action*; Neidle, S., Waring, M. J., Eds.; Verlag Chemie: England, 1983; pp 1−34.

(2) (a) Brãna, M. F.; Castellano, J. M.; Morán, M.; Pérez de Vega, M. J.; Romerdahl, C. R.; Qian, X.-D.; Bousquet, P.; Emling, F.; Schlick, E.; Keilhauer, G. Bis-naphthalimides: a new class of antitumor agents. *Anti-Cancer Drug Des.* **1993**, *8*, 257−268. (b) Thompson, J.; Pratt, C. B.; Stewart, C. F.; Avery, L.; Bowman, L.; Zamboni, W. C.; Pappo, A. Phase I study of DMP 840 in pediatric patients with refractory solid tumors. *Invest. New Drugs* **1998**, *16*, 45−49. (c) Wilson, W. D.; Keel, R. A.; Jones, R. L.; Mosher, C. W. Viscometric analysis of the interaction of bisphenanthridinium compounds with closed circular supercoiled and linear DNA. *Nucleic Acids Res.* **1982**, *10*, 4093−4106. (d) LoRusso, P.; Demchik, L.; Dan, M.; Polin, L.; Gross, J. L.; Corbett, T. H. Comparative efficacy of DMP 840 against mouse and human solid tumor models. *Invest. New Drugs* **1995**, *13*, 195−203.

(3) Garbay-Jaureguiberry, C.; Barsi, M. C.; Jacquemin-Sablon, A.; Le Pecq, J. B.; Roques, B. P. Synthesis and biological activity of new dimers in the 7*H*-pyrido[4,3-*c*]carbazole antitumor series. *J. Med. Chem.* **1992**, *35*, 72−81.

(4) (a) Leng, F.; Priebe, W.; Chaires, J. B. Ultratight DNA binding of a new bisintercalating anthracycline antibiotic. *Biochemistry* **1998**, *37*, 1743−1753. (b) Portugal, J.; Martin, B.; Vaquero, A.; Ferrer, N.; Villamarin, S.; Priebe, W. Analysis of the effects of daunorubicin and WP631 on transcription. *Curr. Med. Chem.* **2001**, *8*, 1−8.

(5) Hernandez, L.; Cholody, W. M.; Hudson, E. A.; Resau, J. H.; Pauly, G.; Michejda, C. J. Mechanism of action of bisimidazoacridones, new drugs with potent, selective activity against colon cancer. *Cancer Res.* **1995**, *55*, 2338−2345. (b) Cholody, W. M.; Kosakowska-Cholody, T.; Michejda, C. J. Bisimidazoacridones induce a potent cytostatic effect in colon tumor cells that sensitizes them to killing by UCN-01. *Cancer Chemother. Pharmacol.* **2001**, *47*, 241−249.

(6) Caubère, P.; Guillaumet, G.; Rodriguez, I.; Vinter-Pasquier, K.; Kuehm-Caubère, C.; Blanchard, S.; Atassi, G.; Pierre, A.; Pfeiffer, B.; Renard, P. Eur. Pat. Appl. EP 9698, 1999; *Chem. Abst.* **2000**, *132*, 22978.

(7) (a) Rodriguez, I.; Kuehm-Caubère, C.; Vinter-Pasquier, K.; Renard, P.; Pfeiffer, B.; Caubère, P. *Tetrahedron Lett.* **1998**, *39*, 7283−7286. (b) Blanchard, S.; Rodriguez, I.; Kuehm-Caubère, C.; Renard, P.; Pfeiffer, B.; Guillaumet, G.; Caubère, P. *Tetrahdron* **2002**, *58*, 3513−3524.

(8) (a) Caubère, P. Applications of sodamide-containing "complex bases" in organic synthesis. *Acc. Chem. Res.* **1974**, *7*, 301−308. (b) Caubère, P. Complex bases and complex reducing agents. New tools in organic synthesis. *Top. Curr. Chem.* **1978**, *73*, 50−124. (c) Caubère, P. Aggregative activation: a helpful concept in organic reactivity and synthesis. *Rev. Heteroat. Chem.* **1991**, *4*, 78−139. (d) Caubère, P. Unimetal super bases. *Chem. Rev.* **1993**, *93*, 2317−2334. (e) Caubère, P. Aggregative activation in connection with a few reactions using transition or alkali metal derivative as intermediates. *J. Chin. Chem. Soc.* **1998**, *45*, 451−463 and references cited in these reviews.

(9) (a) Barlocco, D.; Bergomi, M.; Menta, E.; Palumbo, M.; Cignarella, G. Tricyclic indenopyridazine derivatives: synthesis, cytotoxic and DNA-binding properties. *Recl. Trav. Chem. Pays-Bas* **1996**, *115*, 25−30. (b) Gary, R.; Denny, W. A.; Hansch, C. Comparative QSAR studies on substituted bis-(acridines) and bis-(phenazines)-carboxamides: a new class of anticancer agents. *Bioorg. Med. Chem.* **2000**, *8*, 1835−1839. (c) Spicer, J. A.; Gamage, S. A.; Rewcastle, G. W.; Finlay, G. J.; Bridewell, D. J. A.; Baguley, B. C.; Denny, W. A. Bis(phenazine-1-carboxamides): structure, activity relationships for a new class of dual topoisomerase I/II-directed anticancer drugs. *J. Med. Chem.* **2000**, *43*, 1350−1358.

(10) (a) Gamage, S. A.; Spicer, J. A.; Rewcastle, G. W.; Milton, J.; Sohal, S.; Dangerfield, W.; Mistry, P.; Vicker, N.; Charlton, P. A.; Denny, W. A. Structure−activity relationships for pyrido-, imidazo, pyrazolo-, pyrazino-, and pyrrolo-phenazinecarboxamides as topoisomerase-targeted anticancer agents. *J. Med. Chem.* **2002**, *45*, 740−743. (b) Wang, S.; Miller, W.; Milton, J.; Vicker, N.; Stewart, A.; Charlton, P.; Mistry, P.; Hardick, D.; Denny, W. A. Structure−activity relationships for analogues of the phenazine-based dual topoisomerase I/II inhibitor XR11576. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 415−418.

(11) (a) Caddick, S. Microwave assisted organic reactions. *Tetrahedron* **1995**, *51*, 10403−10432. (b) Loupy, A.; Petit, A.; Hamelin, J.; Texier-Boullet, F.; Jacquault, P.; Mathé, D. New solvent-free organic synthesis using focused microwaves. *Synthesis* **1998**,

1213−1233. (c) Varma, R. S. Solvent-free organic syntheses using supported reagents and microwave irradiation. *Green Chem*. **1999**, 43−55. (d) Finaru, A.; Berthault, A.; Besson, T.; Guillaumet, G.; Berteina-Raboin, S. Microwave-assisted solid-phase synthesis of 5-carboxamido-*N*-acetyltryptamine derivatives. *Org. Lett*. **2002**, *4*, 2613−2615.

(12) Basha, A.; Lipton, M.; Weinreb, S. M. A mild general method for conversion of esters to amides. *Tetrahedron Lett*. **1977**, 4171−4174.

(13) Gilman, N. W. The preparation of carboxylic amides from aldehydes by oxidation. *Chem. Commun*. **1971**, 733−7344.

(14) Jasztold-Howorko, R.; Landras, C.; Pierre, A.; Atassi, G.; Guilbaud, N.; Kraus-Berthier, L.; Léonce, S.; Rolland, Y.; Prost, J. F.; Bisagni, E. Synthesis and evaluation of 9-hydroxy-5-methyl- and (5,6-dimethyl)-6*H*-pyrido[4,3-*b*]carbazole-1-*N*[(dialkylamino)-alkyl]carboxamides a new promising series of antitumor olivacine derivatives. *J. Med. Chem*. **1996**, *37*, 2245−2252.

(15) (a) Bailly, C.; Hénichart, J. P.; Colson, P.; Houssier, C. Drug−DNA sequence-dependent interactions analysed by electric linear dichroism. *J. Mol. Recognit*. **1992**, *5*, 155−171. (b) Colson, P.; Bailly, C.; Houssier, C. Electric linear dichroism as a new tool to study sequence preference in drug binding to DNA. *Biophys. Chem*. **1996**, *58*, 125−140.

(16) (a) Bailly, C.; Braña, M.; Waring, M. J. Sequence-selective intercalation of antitumor bisnaphthalimides into DNA. Evidence for an approach via the major groove. *Eur. J. Biochem*. **1996**, *240*, 195−208. (b) Bailly, C.; Carrasco, C.; Joubert, A.; Bal, C.; Wattez, N.; Hildebrand, M. P.; Lansiaux, A.; Colson, P.; Houssier, C.; Cacho, M.; Ramos, A.; Braña, M. F. Chromophore-modified bis-naphthalimides: DNA recognition, topoisomerase inhibition and cytotoxic properties of two mono- and bis-furonaphthalimides. *Biochemistry* **2003**, *42*, 4136−4150.

(17) Muller, D.; Zeltser, I.; Bitan, G.; Gilon, C. Building units for *N*-backbone cyclic peptides 3. Synthesis of protected N^{α}-(*ω*-aminoalkyl)amino acids and N^{α}-(*ω*-carboxyalkyl)amino acids. *J. Org. Chem*. **1997**, *62*, 411−416.

(18) Urry, W. H.; Szecsi, P.; Ikoku, C.; Moore, D. W. Electrophilic addition reactions of 1,1-dimethyldiazenium bromide with 1,3-dienes and styrenes. *J. Am. Chem. Soc*. **1964**, *86*, 2224−2229.

(19) Attenburrow, J.; Cameron, F. B.; Chapman, J. H.; Evans, R. M.; Hens, B. A.; Jansen, A. B. A.; Walker, T. A synthesis of vitamin A from cyclohexanone. *J. Am. Chem. Soc*. **1952**, 1094−1111.

(20) Pierre, A.; Kraus-Berthier, L.; Atassi, G.; Cros, S.; Poupon, M. F.; Lavielle, G.; Berlion, M.; Bizarri, J. P. Preclinical antitumor activity of a new vinca alkaloid derivative, S12363. *Cancer Res*. **1991**, *51*, 2312−2318.

(21) Goossens, J. F.; Bouey-Bencteux, E.; Houssin, R.; Hénichart, J. P.; Colson, P.; Houssier, C.; Laine, W.; Baldeyrou, B.; Bailly, C. DNA interaction of the tyrosine protein kinase inhibitor PD153035 and its *N*-methyl analogue. *Biochemistry* **2001**, *40*, 4663−4671.

(22) Bailly, C.; Waring, M. J. Comparison of different footprinting methodologies for detecting binding sites for a small ligand on DNA. *J. Biomol. Struct. Dyn*. **1995**, *12*, 869−898.

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