DNA Binding To Guide the Development of Tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Derivatives as Cytotoxic Agents

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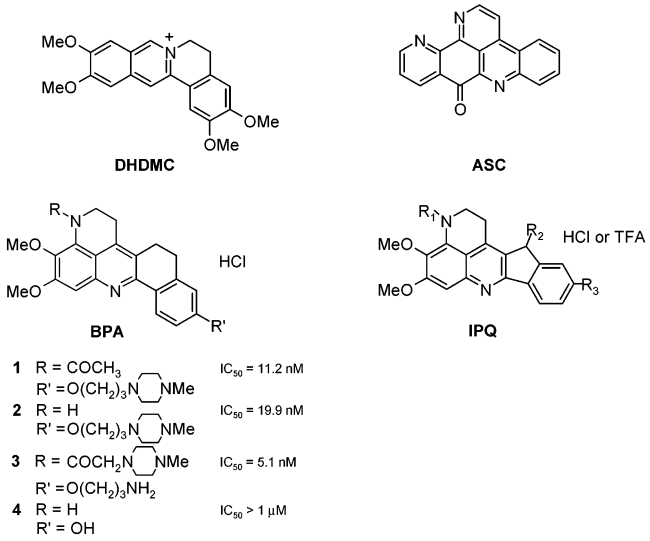
The tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline chromophore was initially designed as a DNA intercalating unit because of its planar structure. Unexpectedly, one molecule (**15d**) bearing two *N*-methylpiperazine chains on both sides of this condensed pentacyclic skeleton fits into the minor groove of DNA and preferentially recognizes AT-rich sequences. The monosubstituted compound **16d** was identified as a potent cytotoxic DNA intercalator, whereas the disubstituted analogue **15d** represents a new structural motif for the development of DNA sequence-reading small molecules.

Introduction

The majority of the drugs used today in the treatment of cancer bind reversibly or irreversibly to DNA or induce DNA damage, either directly or via topoisomerase inhibition. DNA and associated proteins remain valid targets for cancer chemotherapy.[1,2] Novel DNA intercalating (acridines,[3] indolocarbazoles,[4] naphthalimides[5]) and alkylating agents (benzoacronycines,[6] pyrrolobenzodiazepines,[7] distamycin conjugates[8]) are still being developed. DNA, whether with two, three, or four strands, still represents one of the most challenging bioreceptors for small molecules and a target of choice for the control of gene expression.[9] The design of cytotoxic agents interfering with DNA metabolism is actively pursued.

Although it is well established that DNA binding is not sufficient to confer cytotoxic activities, interaction with DNA is often considered as a necessary criterion to maintain a cytotoxic effect, at least for some series of planar intercalating chromophores such as acridines and ellipticines. On the basis of this assumption, we have recently reported the design of DNA-targeted benzo[*c*]pyrido[2,3,4-*kl*]acridines[10] (BPA) combining the structural architecture (Chart 1) of the topoisomerase I inhibitor 5,6-dihydro-8-desmethylcoralyne[11] (DHDMC) and the topoisomerase II poison ascididemin[12] (ASC). The combination of the pyrido[2,3,4-*kl*]acridine skeleton of ASC with the isoquinoline core of DHDMC led to the synthesis of the BPA pentacyclic system,[13] which provided the basis for the development of potent cytotoxic agents. Indeed, among the numerous BPA recently synthesized, we identified three compounds, **1**−**3** (Chart 1), highly toxic to PC 3 prostate cancer cells. These compounds showed little or no effects on the catalytic activities of topoisomerases, but they bound to DNA. We thought that drug−DNA interaction could be used as a guide to develop more active compounds in this series. The BPA chromophore is not planar because of its unsaturated six-membered ring, which introduces a kink in the structure. Considering that a planar structure may be more favorable for stacking interactions between the DNA base pairs, we envisaged the replacement of the six-membered ring with a smaller five-membered ring in order to produce a more flattened conformation. Here, we report the synthesis of a series of tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline derivatives (IPQ) bearing various carbon side chains or

Chart 1



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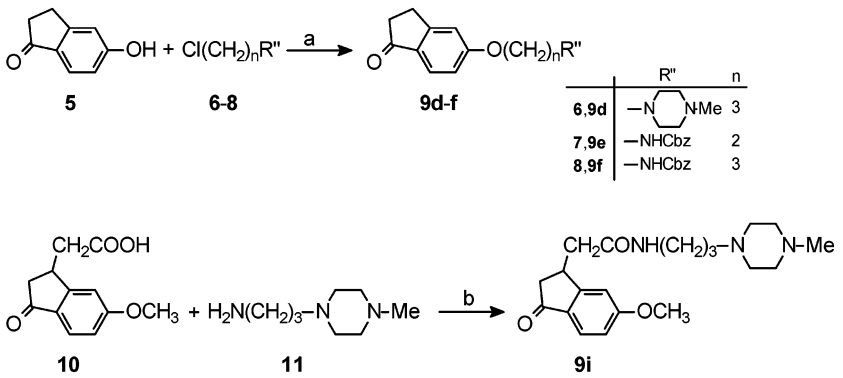
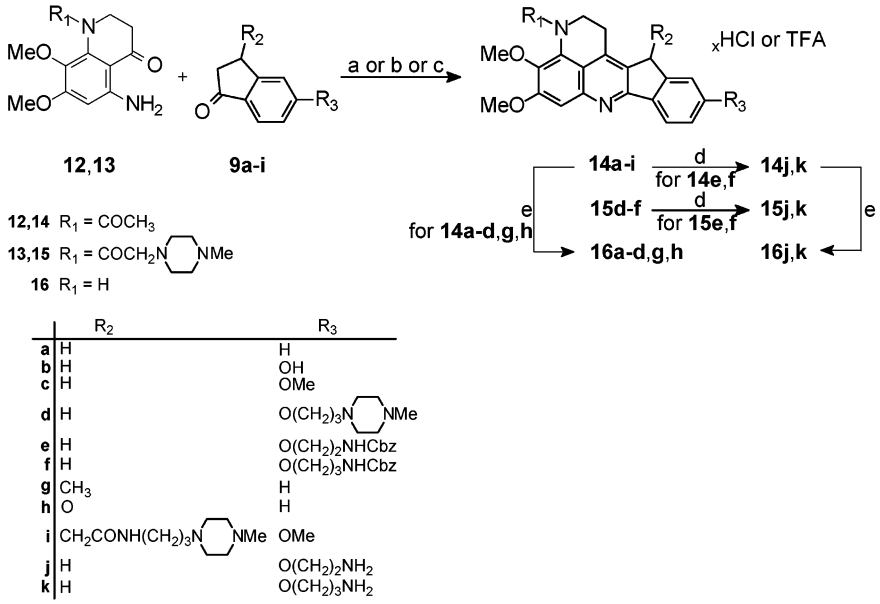
Scheme 1[a]



[a] Reagents and conditions: (a) K_2CO_3, DMF, 80 °C; (b) EDCI, HOBt, NMM, CH_2Cl_2−DMF.

Scheme 2[a]



[a] Reagents and conditions: (a) (i) PPTS, butan-1-ol, reflux, (ii) HCl/MeOH; (b) (i) PPTS, toluene/EtOH (2:1), reflux, (ii) HCl/MeOH; (c) (i) PPTS, toluene, reflux, (ii) TFA; (d) (i) $HCOONH_4$, Pd/C, MeOH, reflux, (ii) HCl/MeOH; (e) (i) 6 N HCl, reflux, (ii) HCl/MeOH.

substituents (Chart 1) corresponding to those of the BPA series. The cytotoxicity of the different compounds was evaluated and their DNA binding strength was measured by complementary biochemical and spectroscopic methods.

Results and Discussion

Chemistry. Indanones **9a**−**c**,**g**,**h** were commercially available, whereas indanones **9d**−**f** were synthesized (Scheme 1) in one step by treating 5-hydroxyindanone **5** with the appropriate *ω*-chloroalkylamines **6**−**8** (protected as carbamates for **7**,**8** in order to prevent imine formation) according to the Williamson conditions (potassium carbonate). Indanone **9i** was obtained from 5-methoxy-1-indanone-3-acetic acid **10** and primary amine **11** using peptidic coupling conditions (EDCI, HOBt).

5-Aminoquinolinones **12** and **13** were synthesized, as previously described,[13] in seven and eight steps, respectively. All of the tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]-quinolines **14a**−**i** and **15d**−**f** were prepared (Scheme 2) by acid-catalyzed Friedländer cyclization[14] between the bicyclic enolizable ketones **9a**−**i** and the substituted *o*-aminoketone **12** or **13**. The structure of indanones **9** is such that only one regioisomer could be formed during the ring closure.

The preparation of **14a**,**c**,**g**,**h** was conveniently carried out using toluene as solvent and pyridinium *p*-toluenesulfonate (PPTS), with distillation of solvent−water azeotrope. In the case of **14d**,**i**, a toluene/ethanol (2:1) mixture was necessary, whereas the reaction leading to **14b** and **15d** was achieved using butan-1-ol. The choice of these different solvents depends on the azeotrope temperature, which must be near 90 °C (to increase the rate and yield of the reaction),[13] and on the solubility of the starting and target molecules in the medium. Condensed pentacycles **14e**,**f** and **15e**,**f** were obtained by reacting aminoketones **12** and **13** with indanones **9e**,**f** in the presence of PPTS and butan-1-ol; the purity of the crude products is sufficient to allow immediately the catalytic cleavage of the Cbz group by ammonium formate−Pd/C and produced **14j**,**k** and **15j**,**k**, respectively. Finally, acid hydrolysis (6 N HCl) of amides **14a**−**d**,**g**,**h**,**j**,**k** yielded the secondary amines **16a**-**d**,**g**,**h**,**j**,**k**.

Biological Data. DNA Interaction. Melting temperature (T_m) and fluorescence measurements were

Table 1. DNA Binding of the IPQ Derivatives

compd	ΔT_m (°C)[a]	K_{app} (10^5 M^{-1})[b]
4	1.1	0.016
14a[c]	nd[d]	nd[d]
14b	3.1	6.77
14c[c]	nd[d]	nd[d]
14d[e]	3.8	35.80
14j[e]	7.0	9.90
14k[e]	6.0	2.93
15d[e]	32.5	30.40
15j[e]	19.6	5.03
15k[e]	22.5	27.80
16a[e]	0	0.031
16b[e]	4.0	6.72
16c[e]	0	0.017
16d[e]	10.0	3.01
16j[e]	14.8	51.40
16k[e]	15.8	61.30

[a] Variation of the ΔT_m ($T_m^{drug-DNAcomplex} - T_m^{DNAalone}$) of the complexes between DNA and the test compounds. [b] Apparent binding constant measured by fluorescence. [c] Trifluoroacetate. [d] nd: not determined. [e] Hydrochloride.

carried out to evaluate the relative DNA binding affinities of the different compounds. T_m analyses were performed with the polynucleotide poly(dAT)$_2$, which melts at a low temperature (42 °C) under our experimental conditions. Calf thymus (CT) DNA, used for the fluorescence assay, melts at a higher temperature (66 °C) and is therefore less convenient in T_m analyses. In nearly all cases, compounds in the designed IPQ series showed superior DNA binding affinities compared to the equivalent BPA compounds. The spectral absorption titrations for **16b** and its analogue **4** in the BPA series showed important spectral changes (marked hypochromism at 360 nm and hyperchromism at 400 nm) but quantitative T_m and fluorescence measurements revealed a higher affinity for the IPQ compound **16b** compared to the BPA analogue **4**. Apparent binding constants were determined by a conventional fluorescence method based on quenching ethidium bromide bound to DNA. The ΔT_m ($T_m^{drug-poly(dAT)_2complex} - T_m^{poly(dAT)_2alone}$) and K_{app} values are collated in Table 1. Compound **16b** in the IPQ series binds to DNA 400 times more strongly than the related BPA compound **4** ($K_{app} = 6.72 \times 10^5$ and 0.016×10^5 M^{-1}, respectively). The difference is less pronounced with compounds bearing one or two cationic side chains, but replacement of the six-membered ring for a five-membered ring almost always reinforces the DNA binding capacity of the molecules. For example, compound **15d** substituted with two *N*-methylpiperazine side chains gave a ΔT_m value of 32.5 °C compared to 9.9 °C for the analogue BPA derivative (compound **3d** in ref 10). Unsurprisingly, the BPA → IPQ conversion reinforces DNA interaction most probably because of the planarity of the IPQ chromophore.

The DNA binding measurements were compared to the cytotoxicity data determined by a colorimetric assay using the prostate PC 3 cell line. Unfortunately, all compounds were found to be noncytotoxic ($IC_{50} > 1$ μM)

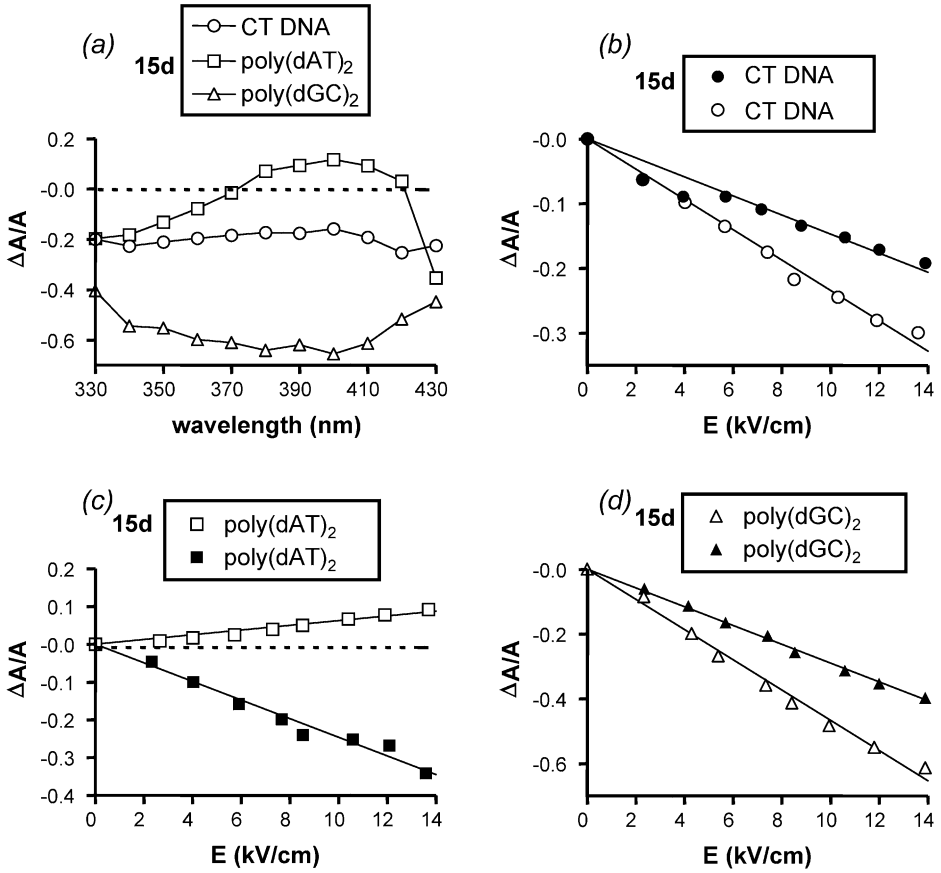


Figure 1. Binding mode of **15d** to DNA studied by electric linear dichroism. (a) ELD spectrum of **15d** bound to (○) calf thymus DNA, (□) poly(dAT)$_2$, and (△) poly(dGC)$_2$. Dependence of the reduced dichroism ΔA/A on electric field strength for the binding of **15d** to (b) calf thymus DNA, (c) poly(dAT)$_2$, and (d) poly(dGC)$_2$. Conditions are as follows: (a) 13.6 kV/cm, $P/D = 25$ (250 μM DNA, 10 μM drug), (b, c, d) $P/D = 25$, 410 nm for the DNA−drug complexes (open symbols) and 260 nm for DNA alone (filled symbols) in 1 mM sodium cacodylate buffer, pH 7.0.

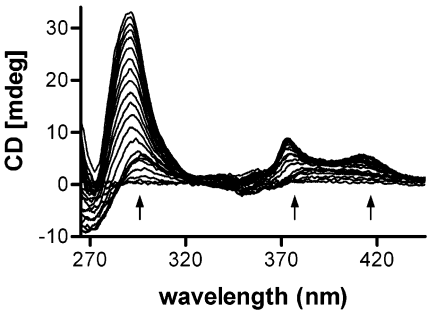


Figure 2. Circular dichroism spectral titration of **15d** with poly(dAT)$_2$. Strong induced signals are observed with increasing ratios of compound to DNA at 290, 375, and 415 nm. The ratios of compounds to DNA (nucleotide) increase from 0 to 10 (bottom to top lines at 295 nm).

with the unique exception of compound **16d** substituted with an *N*-methylpiperazine side chain, which maintained high antiproliferative activity. It is also worth mentioning that these compounds, as in the BPA series, showed no effect on DNA topoisomerases I or II. Of the three active compounds **1**−**3** identified in the BPA series (Chart 1), only one remains active in the IPQ series, **16d** (IC_{50} = 6.5 nM), which has the same substitution pattern as **2** (IC_{50} = 19.9 nM). The slight gain in activity may be due to its improved DNA binding capacity, but there is no direct correlation between DNA binding and cytotoxicity. In the IPQ series, some of the compounds, such as **15d**, exhibit a high affinity for DNA, but this improved binding is not reflected in cytotoxic potential. The substitution of the pentacyclic ring does not confer superior activity in terms of DNA binding or cytotoxicity. Therefore, it seems reasonable to leave this position unsubstituted.

All the BPA and IPQ compounds are supposed to intercalate into DNA. The binding mode was studied by electric linear dichroism. This electro-optical method has proved to be most useful in determining the orientation of drugs bound to DNA. An additional advantage is that it senses only the orientation of the polymer-bound ligand; free ligand is isotropic and does not contribute to the signal.[15] Negative reduced dichro-

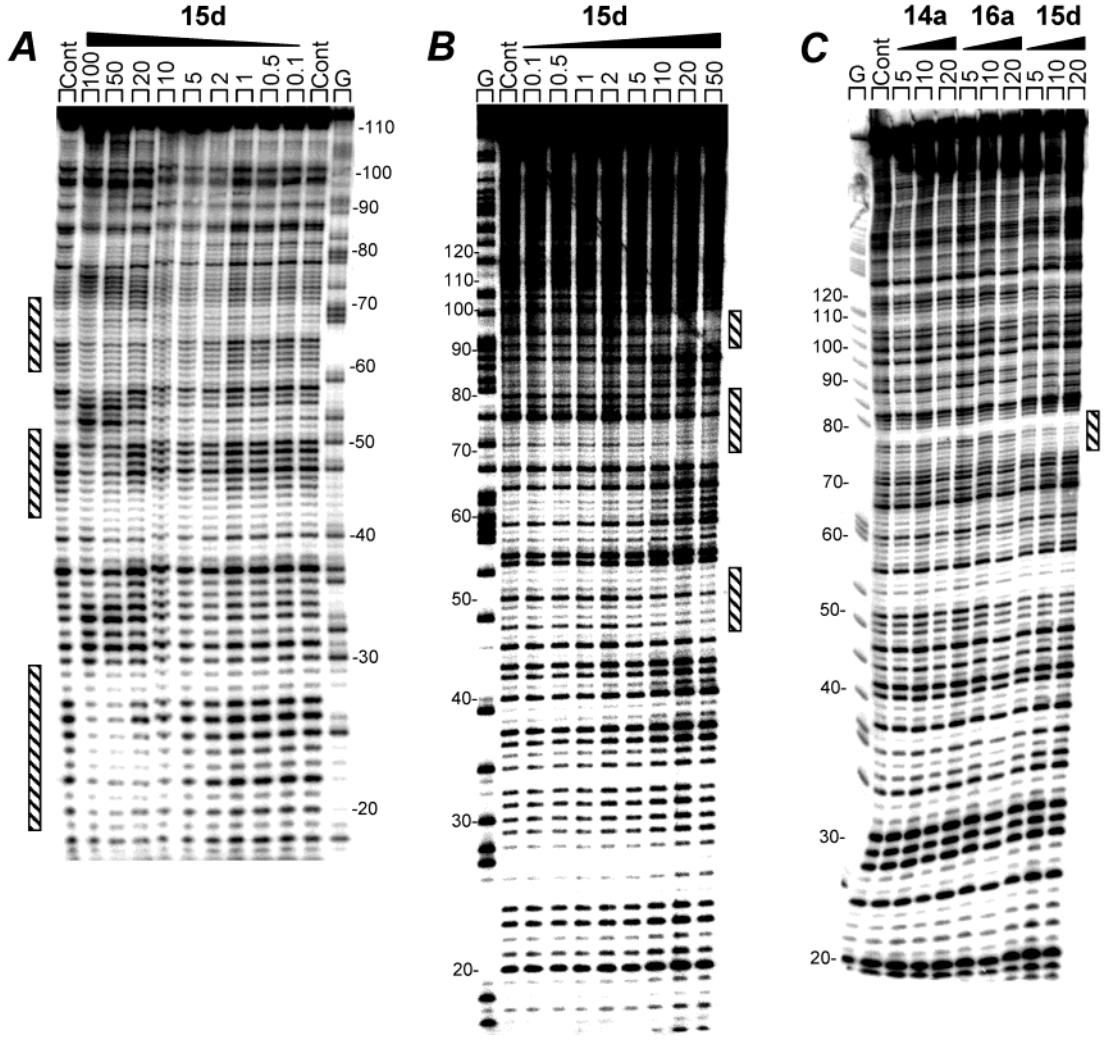


Figure 3. Sequence selective binding of **15d**, **14a**, **16a** (micromolar concentrations). The gels show DNase I footprinting with two DNA restriction fragments of (A) 117, (B) 198, and (C) 265 base pairs. The 117-mer PvuII-EcoRI fragment was cut from the plasmid pBS. The 198-mer HindIII-XbaI fragment was obtained from plasmid pMS1. In each case, DNA was 3′-end-labeled at the EcoRI or HindIII 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 (marked Cont) contained no drug. Guanine-specific sequence markers obtained by treatment of DNA with dimethyl sulfate followed by piperidine were run in the lanes marked G. Numbers on the side of the gels refer to the standard numbering scheme for the nucleotide sequence of the DNA fragment.

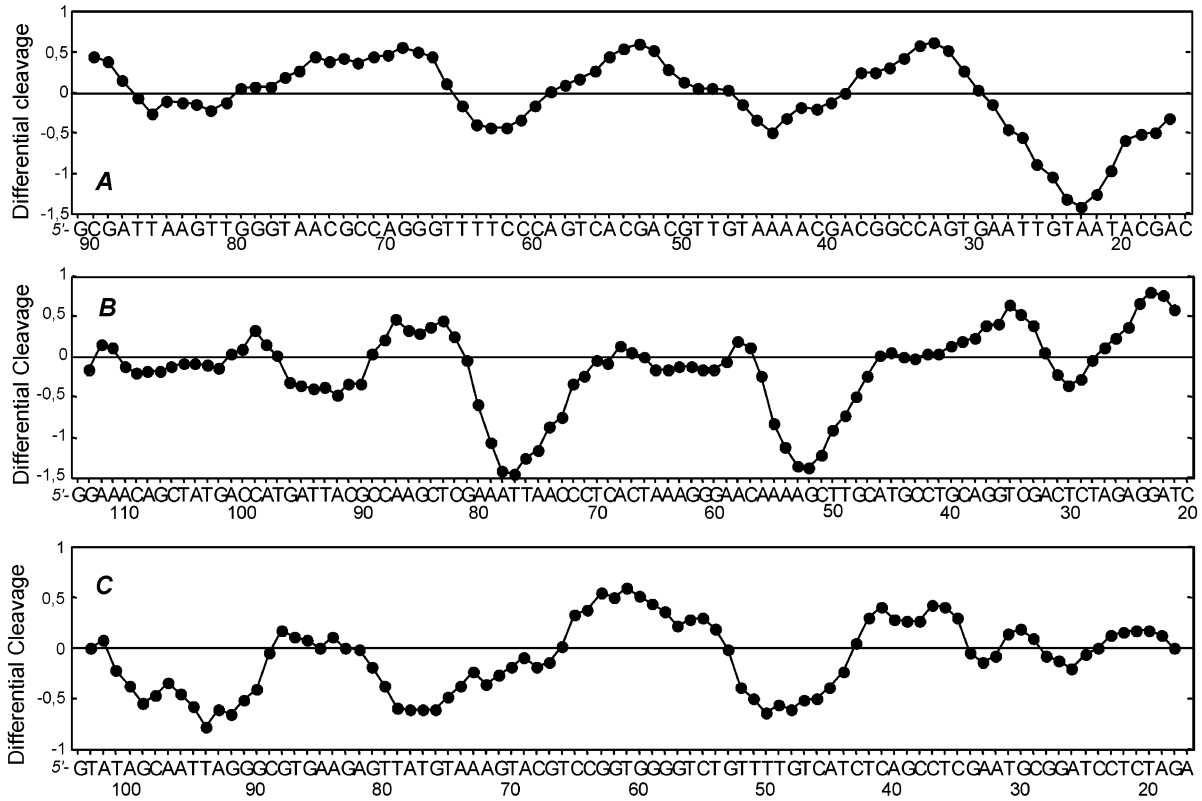


Figure 4. Differential cleavage plots comparing the susceptibility of the three DNA fragments of (A) 117, (B) 265, and (C) 198 base pairs to DNase I cutting in the presence of **15d** at 20 μM. Negative values correspond to a ligand-protected site, and positive values represent enhanced cleavage. Vertical scales are in units of ln(fa) − ln(fc), where fa is the fractional cleavage at any bond in the presence of the drug and fc is the fractional cleavage of the same bond in the control, given closely similar extents of overall digestion. Each line drawn represents a three-bond running average of individual data points, calculated by averaging the value of ln(fa) − ln(fc) at any bond with those of its two nearest neighbors. Only the region of the restriction fragments analyzed by densitometry is shown.

ism ($\Delta A/A$) signals were recorded with all the IPQ compounds bound to calf thymus DNA, and this behavior is typical of intercalating agents. However, here again we observed an unusual effect with one molecule, the bis-*N*-methylpiperazine derivative **15d**. This compound (Figure 1) gave reduced $\Delta A/A$ signals with calf thymus DNA and poly(dGC)$_2$ but positive signals with poly(dAT)$_2$.

Positive ELD data are commonly obtained with minor groove binders that are positioned at about 45° relative to the long axis of the DNA molecule (i.e., the electric field direction).[15] The binding mode of **15d** was investigated further by circular dichroism. The CD spectral titration for **15d** (Figure 2) with poly(dAT)$_2$ (ratios **15d**/DNA ranging from 0 to 10) gives a strong induced CD signal in the compound absorption region above 300 nm and large changes in the DNA absorption region. Positive CD and ELD values with the AT DNA are consistent with a dominant minor groove binding mode for **15d**.

All newly synthesized compounds have been tested for DNA binding by spectroscopic and footprinting methods. None of the intercalating agents give any footprint, whereas the minor groove binder **15d** was shown to protect DNA from cleavage by Dnase I at certain sites. This minor groove mode binder **15d** recognizes preferentially AT-rich DNA sequences (Figure 3), as revealed by the DNase I footprinting experiments performed with three different DNA radiolabeled fragments of 117-bp, 178-bp, and 265-bp. It is clear that compound **15d** protects certain sequences from cleavage by the nuclease, and the densitometric analyses of the gels indicate that the protected sequences mainly correspond to AT sites. The differential cleavage plots indicate (Figure 4) selective binding of **15d** to the following sites: 5′-TAATA, 5′-TAAAA, 5′-TTTT, 5′-ATTAA, and 5′-AAATTAA.

Footprints were detected only with **15d**, the other compounds showing no inhibition of DNase I cleavage. The compounds having no side chain (**16a**, **16b**) or only one side chain (**14d**, **16d**) intercalate into DNA and have no sequence preference. The incorporation of a second cationic side chain confers AT sequence recognition by virtue of the minor groove binding mode. In other words, the substitution of the quinoline nitrogen by an *N*-methylpiperazine chain changes the intercalative binding mode of **16d** for a minor groove binding process detected with **15d**.

Conclusions

We have designed a new DNA binding unit, the tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline (IPQ) chromophore, which is derived from the benzo[*c*]pyrido-[2,3,4-*kl*]acridine (BPA) pentacyclic system recently studied.[10,13] The IPQ system is more planar than the BPA system, and consequently, binding to DNA is generally reinforced. These two pentacyclic heterocycles perform more or less efficiently in terms of cytotoxicity. No direct relationship between DNA binding strength

and cytotoxicity to PC 3 prostate cancer cells can be established, but a few highly cytotoxic molecules have been identified in both the BPA and IPQ series. These compounds were initially designed as DNA intercalating agents, but a compound that fits into the minor groove of AT-rich DNA sequences has been identified in the IPQ series. The extended molecule **15d** bearing two *N*-methylpiperazine chains on both sides of the IPQ chromophore provides a new motif for duplex DNA recognition but also for more complex nucleic acid structures. Indeed, recent preliminary studies (unpublished data) reveal that this molecule binds to the telomer quadruplex sequence $AG_3(TTAG_3)_3$ and exhibits a slight inhibitory activity against human telomerase. This novel activity opens new perspectives for the design of antitumor agents in this series.

Experimental Section

Chemistry. Analytical thin-layer chromatography (TLC) was performed on precoated Kieselgel 60F_{254} plates (Merck). Compounds were visualized by UV and/or with iodine, and R_f are given for guidance. Melting points were determined with a capillary melting point apparatus and remain uncorrected. The structures of all compounds were supported by IR (KBr pellets, FT-Bruker Vector 22 instrument) and ^{1}H NMR at 300 MHz on a Bruker DPX-300 spectrometer. Chemical shifts δ are reported in ppm downfield from tetramethylsilane, J values are in hertz, and the splitting patterns were designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. Electron impact (EI) mass spectra were obtained on a quadripolar Finningan Mat SSQ710 spectrometer. Elemental analyses were performed by the "Service Central d'Analyses" at the CNRS, Vernaison (France). Dimethylformamide was distilled from CaH_2 and stored over 3Å molecular sieves.

Materials. The preparation of aminoketones **12** and **13** has been previously described.[13] Compounds **9a**−**c**,**g**,**h**, **10**, and PPTS were purchased from Sigma Aldrich (Saint Quentin Fallavier, France).

5-[3-(4-Methyl)piperazin-1-ylpropoxy]indan-1-one (9d). Potassium carbonate (3.73 g, 27 mmol) was added to a solution of 5-hydroxyindanone **5** (1 g, 6.7 mmol) in dry DMF (10 mL), and the mixture was stirred for 30 min at room temperature. Then 1-(3-chloropropyl)-4-methylpiperazine dihydrochloride (1.35 g, 5.4 mmol) was added. The mixture was stirred for 3 h at 80 °C, then diluted with water and extracted several times with ethyl acetate. The organic layers were washed with a 10% potassium carbonate solution and a saturated aqueous sodium chloride solution and dried over magnesium sulfate. Concentration followed by column chromatography (silica gel, 15% methanol−ammoniacal dichloromethane) gave 940 mg (60% yield) of **9d** as a brown oil: R_f = 0.62 (CH_2Cl_2/MeOH 85:15, NH_3); IR 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.97 (p, J = 6.4, 2H), 2.27 (s, 3H), 2.49−2.66 (m, 12H), 3.05 (t, J = 6.4, 2H), 4.06 (t, J = 6.4, 2H), 6.83 (dd, J = 2.0, J = 7.3, 1H), 6.88 (d, J = 2.0, 1H), 7.64 (d, J = 7.3, 1H).

General Procedure for the Synthesis of Compounds 9e,f. Potassium carbonate (5.6 g, 40 mmol) was added to a solution of 5-hydroxyindanone **5** (3 g, 20 mmol) in dry DMF (20 mL), and the mixture was stirred for 30 min at room temperature. Then the appropriate ω-chloroalkylcarbamate **7**[16] or **8**[17] (22 mmol) was added. The mixture was stirred for 3 h at 80 °C, then diluted with water and extracted several times with ethyl acetate. The organic layers were washed with saturated aqueous sodium chloride and dried over magnesium sulfate. Concentration followed by column chromatography (silica gel, 50% heptane−ethyl acetate) and trituration in isopropyl ether gave 3.3 g (50% yield) of **9e** or 4.6 g (66% yield) of **9f** as white powders.

5-[2-(*N*-Benzyloxycarbonyl)aminoethoxy]indan-1-one (9e). Mp 72 °C (EtOH/H_2O); R_f = 0.62 (CH_2Cl_2/MeOH 9:1); IR 1702, 3331 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.66 (t, J = 6.1, 2H), 3.07 (t, J = 6.1, 2H), 3.65 (q, J = 5.5, 2H), 4.11 (t, J = 5.2, 2H), 5.12 (s, 2H), 5.33 (bs, 1H), 6.86 (s, 1H), 6.88 (d, J = 6.6, 1H), 7.35 (s, 5H), 7.67 (d, J = 9.2, 1H).

5-[3-(*N*-Benzyloxycarbonyl)aminopropoxy]indan-1-one (9f). Mp 93 °C (EtOH/H_2O); R_f = 0.70 (CH_2Cl_2/MeOH 9:1); IR 1685, 1716, 3298 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (p, J = 5.6, 2H), 2.67 (t, J = 6.0, 2H), 3.08 (t, J = 5.6, 2H), 3.43 (p, J = 6.0, 2H), 4.10 (t, J = 5.6, 2H), 5.01 (bs, 1H), 5.11 (s, 2H), 6.88−6.90 (m, 2H), 7.35 (s, 5H), 7.68 (d, J = 9.3, 1H).

2-(6-Methoxy-2-oxo-2,3-dihydro-1*H*-1-indenyl)-*N*-[3-(4-methylpiperazino)propyl]acetamide (9i). EDCI (530 mg, 3.4 mmol), HOBt (460 mg, 3.4 mmol), and 4-methylmorpholine (0.38 mL, 3.4 mmol) were added to a solution of 5-methoxy-1-indanone-3-acetic acid (500 mg, 2.2 mmol) in 10 mL of a mixture of dichloromethane/dimethylformamide (1:1). After agitation for 1 h at room temperature, 1-(3-aminopropyl)-4-methylpiperazine (360 mg, 2.2 mmol) was added and the mixture was stirred for another 12 h. The residue obtained after evaporation of the solvents was dissolved in dichloromethane before extraction with a 10% potassium carbonate solution. The organic layer was washed with a saturated aqueous sodium chloride solution and dried over magnesium sulfate. Concentration followed by column chromatography (silica gel, 70% dichloromethane−ammoniacal methanol) gave 561 mg (71% yield) of **9i** as a brown oil. R_f = 0.33 (CH_2Cl_2/MeOH 7:3, NH_3); IR 1648, 1704, 3313 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.63 (p, J = 6.2, 2H), 2.19 (s, 3H), 2.27−2.43 (m, 10H), 2.52 (dd, J = 6.5, J = 7.8, 2H), 2.90 (dd, J = 7.4, J = 7.8, 1H), 3.21 (bs, 2H), 3.32 (q, J = 5.8, 2H), 3.40 (s, 3H), 6.87 (dd, J = 2.3, J = 8.4, 1H), 6.90 (d, J = 2.3, 1H), 7.44 (t, 1H), 7.62 (d, J = 8.4, 1H). Anal. ($C_{20}H_{29}N_3O_3$) C, H, N.

General Procedure for the Synthesis of Compounds 14a,c,g,h. A solution of **12** (500 mg, 1.89 mmol), PPTS (470 mg, 1.89 mmol), and appropriate indanone **9a**,**c**,**g**, or **h** (2.84 mmol) in toluene (10 mL) was heated at reflux for 24 h using a Dean−Stark trap. The precipitate obtained after cooling was collected by filtration and washed successively with toluene and ether. The crude product was subjected to column chromatography (silica gel, 97% dichloromethane−methanol−TFA (5 drops)). The solid was then recrystallized.

3-Acetyl-4,5-dimethoxy-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Trifluoroacetate (14a). Yellow solid (340 mg, 50% yield); R_f = 0.58 (CH_2Cl_2/MeOH 9:1); mp 247 °C (EtOH 95%); IR 1609, 1667 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3H), 3.36−3.51 (m, 3H), 3.99 (s, 3H), 4.12 (s, 2H), 4.14 (s, 3H), 5.32 (bs, 1H), 7.51 (s, 1H), 7.65−7.80 (m, 3H), 8.25 (d, J = 8.0, 1H); MS (EI) m/z 360 (M^+, 81), 318 (37), 303 (100). Anal. ($C_{22}H_{20}N_2O_3$·TFA·0.5H_2O) C, H, N.

3-Acetyl-4,5,10-trimethoxy-1,2,3,12-tetrahydroindeno-[1,2-*b*]pyrido[4,3,2-*de*]quinoline Trifluoroacetate (14c). Brilliant-yellow solid (317 mg, 43% yield); R_f = 0.45 (CH_2Cl_2/MeOH 9:1); mp >250 °C (EtOH 95%); IR 1607, 1667 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H), 3.29−3.45 (m, 3H), 3.97 (s, 3H), 3.99 (s, 3H), 4.04 (s, 2H), 4.11 (s, 3H), 5.33 (bs, 1H), 7.18 (d, J = 9.1, 1H), 7.25 (s, 1H), 7.44 (s, 1H), 8.17 (d, J = 8.7, 1H); MS (EI) m/z 390 (M^+, 44), 348 (10), 333 (100). Anal. ($C_{23}H_{22}N_2O_4$·TFA·0.5H_2O) C, H, N.

3-Acetyl-4,5-dimethoxy-12-methyl-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Trifluoroacetate (14g). Light-yellow solid (590 mg, 71% yield); R_f = 0.45 (CH_2Cl_2/MeOH 9:1); mp >250 °C (EtOH 95%); IR 1608, 1667 cm^{-1}; ^{1}H NMR ($CDCl_3$, TFA-d_1) δ 2.27 (s, 3H), 3.32−3.48 (m, 3H), 3.94 (s, 3H), 3.99 (s, 3H), 4.01 (s, 1H), 4.15 (s, 3H), 5.33 (bs, 1H), 7.32 (dd, J = 2.3, J = 8.3, 1H), 7.66 (t, J = 8.8, 2H), 7.70 (s, 1H), 7.87 (dd, J = 2.3, J = 8.3, 1H); MS (EI) m/z 374 (M^+, 52), 332 (34), 317 (100). Anal. ($C_{23}H_{22}N_2O_3$·TFA·0.5H_2O) C, H, N.

3-Acetyl-4,5-dimethoxy-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinolin-12-one Trifluoroacetate (14h). Yellow solid (533 mg, 62% yield); R_f = 0.40 (CH_2Cl_2/MeOH 9:1); mp 213 °C (EtOH 95%); IR 1612, 1664, 1707 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3H), 3.24−3.33 (m, 2H), 3.69 (bs, 1H), 3.79 (s, 3H), 4.01 (s, 3H), 5.06 (bs, 1H), 7.31 (s, 1H), 7.42 (t, J =

7.6, 1H), 7.69 (d, J = 7.6, 1H), 7.78 (t, J = 7.6, 1H), 7.93 (d, J = 7.6, 1H); MS (EI) m/z 374 (M^+, 84), 332 (52), 317 (100). Anal. ($C_{22}H_{18}N_2O_4 \cdot 0.5TFA \cdot 0.5H_2O$) C, H, N.

3-Acetyl-10-hydroxy-4,5-dimethoxy-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline (14b). A solution of **12** (500 mg, 1.89 mmol), PPTS (710 mg, 2.84 mmol), and indanone **9b** (840 mg, 5.68 mmol) in butan-1-ol (10 mL) was heated under reflux for 3 h using a Dean−Stark trap. After the mixture cooled, the solvent was evaporated and the residue was triturated with ether. After the precipitate was washed with ether, the crude product was subjected to column chromatography (silica gel, 95% dichloromethane−methanol). Evaporation of the solvents gave 534 mg (75% yield) of pure **14b** as a pale-yellow solid. Mp >250 °C (MeOH/Et_2O); R_f = 0.45 (CH_2Cl_2/MeOH 9:1); IR 1649, 1667, 3423 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.28 (s, 3H), 3.29 (bs, 2H), 3.63 (bs, 1H), 3.84 (s, 3H), 4.01 (s, 2H), 4.05 (s, 3H), 4.63 (bs, 1H), 7.00 (d, J = 8.0, 1H), 7.10 (s, 2H), 7.13 (s, 1H), 8.12 (d, J = 8.4, 1H), 10.81 (bs, 1H); MS (EI) m/z 376 (M^+, 43), 334 (64), 319 (100). Anal. ($C_{22}H_{20}N_2O_4 \cdot 2.25H_2O$) C, H, N.

3-Acetyl-4,5-dimethoxy-10-[3-(4-methyl)piperazin-1-ylpropoxy]-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Trihydrochloride (14d). A solution of **12** (2 g, 7.57 mmol), PPTS (950 mg, 3.79 mmol), and indanone **9d** (1.09 g, 3.79 mmol) in 15 mL of a mixture of toluene/ethanol (2:1) was heated under reflux for 5 days using a Dean−Stark trap. The precipitate formed after cooling to room temperature was collected by filtration and washed successively with toluene and ether. The crude product was subjected first to column chromatography (silica gel, 90% dichloromethane−methanol), then to preparative HPLC (C_{18}, 4.6 mm × 150 mm, 5 μm, 100 Å, 34% MeOH−66% H_2O−0.1% TFA). Evaporation of the appropriate fraction (t_R = 15.05 min) gave 710 mg (42% yield) of a beige solid. Methanol saturated with HCl was added to a methanolic solution of this solid to yield **14d** as a pale-yellow powder. Mp 250 °C (EtOH/Et_2O); R_f = 0.12 (CH_2Cl_2/MeOH 9:1, NH_3); IR 1647, 1670 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.02 (s, 3H), 2.61−3.67 (m, 18H), 3.83 (s, 3H), 4.03 (s, 3H), 4.08 (s, 2H), 4.21 (bt, 2H), 4.63 (bs, 1H), 7.18 (d, J = 8.6, 1H), 7.29 (s, 1H), 7.61 (s, 1H), 8.42 (d, J = 8.6, 1H); MS (EI) m/z 516 (M^+, 72), 446 (40), 388 (20), 376 (100), 141 (78), 113 (69), 43 (92). Anal. ($C_{30}H_{36}N_4O_4 \cdot 3HCl \cdot 5H_2O$) C, H, N, Cl.

2-(3-Acetyl-4,5,10-trimethoxy-1,2,3,12-tetrahydroindeno-[1,2-*b*]pyrido[4,3,2-*de*]quinolin-12-yl)-*N*-[3-(4-methylpiperazino)propyl]acetamide Trihydrochloride (14i). Compound **14i** was prepared from **12** and **9i** according to the procedure described for **14d**. Methanol saturated with HCl was added to a methanolic solution of the crude material to yield **14i** as a pale-yellow powder (1.55 g, 54% yield). Mp 225 °C (EtOH/Et_2O); R_f = 0.15 (CH_2Cl_2/MeOH 9:1, NH_3); IR 1645, 3422 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.02 (s, 3H), 2.61−3.67 (m, 20H), 3.83 (s, 3H), 3.91 (s, 3H), 4.03 (s, 3H), 4.70 (s, 1H), 4.95 (bs, 1H), 7.18 (d, J = 8.6, 1H), 7.24 (s, 1H), 7.89 (s, 1H), 8.63 (d, J = 8.6, 1H), 11.98 (bs, 1H); MS (EI) m/z 588 (M + 1, 5), 586 (48), 531 (100), 419 (14), 391 (91), 332 (33), 276 (58), 207 (42). Anal. ($C_{33}H_{41}N_5O_5 \cdot 3HCl \cdot 3.5H_2O$) C, H, N, Cl.

General Procedure for the Synthesis of Compounds 14j,k. A solution of **12** (500 mg, 1.89 mmol), PPTS (710 mg, 2.84 mmol), and the appropriate indanone **9e** or **9f** (5.68 mmol) in butan-1-ol (10 mL) was heated under reflux for 5 h using a Dean−Stark trap. After the mixture cooled, the solvent was evaporated and the residue was triturated with ether. After the precipitate was washed with ether, the product was subjected to column chromatography (silica gel, 97% dichloromethane−methanol). The crude solid **14e** or **14f** obtained after evaporation of the solvents was dissolved in MeOH (10 mL), and Pd on C was added. The mixture was heated at reflux for 30 min before addition of ammonium formate (10 equiv). After 2 h, the warmed solution was filtered through Celite and washed with MeOH, and the filtrate was evaporated. The concentrate was subjected to column chromatography (silica gel, 20% methanol−1% ammoniacal dichloromethane). Evaporation of the solvents gave respectively 436 mg (55% yield) or 344 mg (42% yield) of pure bases. Methanol saturated with HCl was added to a solution of these solids in methanol to yield **14j** or **14k** as yellow solids that were then recrystallized.

3-Acetyl-10-(2-aminoethoxy)-4,5-dimethoxy-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Dihydrochloride (14j). Yellow solid; mp >250 °C (MeOH/Et_2O); R_f = 0.46 (CH_2Cl_2/MeOH 9:1, NH_3); IR 1617, 1645, 3423 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.09 (s, 3H), 3.33−3.39 (m, 4H), 3.60 (bs, 1H), 3.83 (s, 3H), 4.04 (s, 3H), 4.13 (s, 2H), 4.35 (t, J = 4.7, 2H), 4.61 (bs, 1H), 7.25 (d, J = 8.7, 1H), 7.43 (s, 1H), 7.86 (s, 1H), 8.31 (bs, 3H), 8.67 (d, J = 8.7, 1H); MS (EI) m/z 419 (M^+, 80), 362 (48), 319 (100). Anal. ($C_{24}H_{25}N_3O_4 \cdot 2HCl \cdot 0.5H_2O$) C, H, N, Cl.

3-Acetyl-10-(3-aminopropoxy)-4,5-dimethoxy-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Dihydrochloride (14k). Yellow solid; mp >250 °C (MeOH/Et_2O); R_f = 0.56 (CH_2Cl_2/MeOH 9:1, NH_3); IR 1645, 1673, 3421 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.10 (s, 5H), 2.99 (bs, 2H), 3.30−3.36 (m, 3H), 3.82 (s, 3H), 4.04 (s, 3H), 4.10 (s, 2H), 4.20 (t, J = 4.7, 2H), 4.61 (bs, 1H), 7.16 (d, J = 7.6, 1H), 7.35 (s, 1H), 7.64 (s, 1H), 8.13 (bs, 3H), 8.43 (d, J = 8.2, 1H); MS (EI) m/z 433 (M^+, 100), 376 (100), 319 (60). Anal. ($C_{25}H_{27}N_3O_4 \cdot 2HCl \cdot 2.5H_2O$) C, H, N, Cl.

4,5-Dimethoxy-3-[2-(4-methyl)piperazin-1-ylacetyl]-10-[3-(4-methyl)piperazin-1-ylpropoxy]-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Pentahydrochloride (15d). A solution of **13** (500 mg, 1.38 mmol), PPTS (520 mg, 2.07 mmol), and indanone **9d** (1.19 g, 4.14 mmol) in butan-1-ol (10 mL) was heated under reflux for 12 h using a Dean−Stark trap. The mixture was cooled to room temperature, made basic with 10% aqueous potassium carbonate, and diluted with ethyl acetate, and the layers were separated. The dark-red organic layer was washed with saturated aqueous sodium chloride and dried over magnesium sulfate. Removal of the solvent gave an oily product that precipitated in ether. After filtration and washing with ether, the crude product was subjected to column chromatography (silica gel, 10% methanol−dichloromethane to 50% methanol−1% ammoniacal dichloromethane). Evaporation of the appropriate fraction gave 659 mg (63% yield) of pure base. Dichloromethane saturated with HCl was added to a solution of the base in dichloromethane to yield **15d** as a yellow solid. Mp >250 °C (EtOH/Et_2O/H_2O); R_f = 0.63 (CH_2Cl_2/MeOH 7:3, NH_3); IR 1644, 1684 cm^{-1}; ^1H NMR, insufficiently soluble in usual solvents; MS (EI) m/z 614 (M^+, 5), 583 (6), 113 (100). Anal. ($C_{35}H_{46}N_6O_4 \cdot 5HCl \cdot 8H_2O$) C, H, N, Cl.

General Procedure for the Synthesis of Compounds 15j,k. A solution of **13** (500 mg, 1.38 mmol), PPTS (520 mg, 2.07 mmol), and the appropriate indanone **9e** or **9f** (4.14 mmol) in butan-1-ol (10 mL) was heated under reflux for 12 h using a Dean−Stark trap. The mixture was cooled to room temperature, made basic with 10% aqueous potassium carbonate, and diluted with ethyl acetate, and the layers were separated. The dark-red organic layer was washed with saturated aqueous sodium chloride and dried over magnesium sulfate. Removal of the solvent gave an oily product that precipitated in ether. After filtration and washing with ether, the crude product corresponding to **15e** or **15f** was subjected to chromatography (silica gel, 95% dichloromethane−methanol). The solid obtained after evaporation of the solvents was dissolved in MeOH (10 mL), and Pd on C was added. The mixture was heated to reflux for 30 min before ammonium formate (10 equiv) was added. After 2 h, the warmed solution was filtered through Celite and washed with MeOH and the filtrate was evaporated. The concentrate was subjected to column chromatography (silica gel, 30% methanol−1% ammoniacal dichloromethane). Evaporation of the solvents gave 414 mg (58% yield) or 403 mg (55% yield) of pure solids. Methanol saturated with HCl was added to a solution of these solids in methanol to yield **15j** or **15k** as yellow powders that were then recrystallized.

10-(2-Aminoethoxy)-4,5-dimethoxy-3-[2-(4-methyl)piperazin-1-yl]-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Trihydrochloride (15j). Yellow solid; mp >250 °C (MeOH/Et_2O/H_2O); R_f = 0.77 (CH_2Cl_2/MeOH 7:3, NH_3); IR 1643, 1682, 3423 cm^{-1}; ^1H NMR (DMSO-d_6/60 °C) δ

2.71 (s, 3H), 3.27−3.39 (m, 12H), 3.82 (s, 3H), 4.02−4.05 (m, 6H), 4.09 (s, 2H), 4.40 (bt, J = 5.2, 3H), 7.10 (s, 1H), 7.12 (dd, J = 2.1, J = 8.6, 1H), 8.01 (s, 1H), 8.41 (bs, 3H), 8.72 (d, J = 8.6, 1H); MS (EI) m/z 517 (M^+, 12), 485 (20), 113 (100), 70 (82). Anal. ($C_{29}H_{35}N_5O_4$·3.75HCl·5H_2O) C, H, N, Cl.

10-(3-Aminopropoxy)-4,5-dimethoxy-3-[2-(4-methyl)-piperazin-1-yl]-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido-[4,3,2-*de*]quinoline Trihydrochloride (15k). Yellow solid; mp >250 °C (MeOH/Et_2O/H_2O); R_f = 0.76 (CH_2Cl_2/MeOH 7:3, NH_3); IR 1642, 1681, 3423 cm^{-1}; ^{1}H NMR (DMSO-d_6/60 °C) δ 2.15 (p, J = 6.5, 2H), 2.71 (s, 3H), 3.00 (bs, 2H), 3.27−3.39 (m, 10H), 3.81−3.86 (m, 6H), 4.01 (s, 3H), 4.09 (s, 2H), 4.23 (bt, J = 6.5, 3H), 7.08 (s, 1H), 7.14 (dd, J = 2.1, J = 7.6, 1H), 7.99 (s, 1H), 8.13 (bs, 3H), 8.55 (d, J = 7.6, 1H); MS (EI) m/z 531 (M^+, 2), 499 (6), 113 (100), 70 (32). Anal. ($C_{30}H_{37}N_5O_4$·3.75HCl·8H_2O) C, H, N, Cl.

General Procedure for Hydrolysis of Amides 14a−d,g,h,j,k. A solution of 500 mg of quinolines **14** in 10 mL of aqueous 6 N HCl was refluxed for 4 h. After cooling to room temperature, the mixture was made basic using 10% aqueous potassium carbonate and extracted with ethyl acetate. The red organic layer was washed with saturated aqueous sodium chloride and dried over magnesium sulfate. After removal of the solvent, the crude oily product was subjected to column chromatography: silica gel; 98% dichloromethane−methanol (**14a**,**c**,**g**,**h**), 97% dichloromethane−methanol (**14b**), 10% methanol−1% ammoniacal dichloromethane (**14d**,**j**,**k**). Evaporation of the solvents gave pure bases. Methanol saturated with HCl was added to a solution of these solids in methanol to yield secondary amines **16** as hydrochlorides, which were then recrystallized.

4,5-Dimethoxy-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido-[4,3,2-*de*]quinoline Hydrochloride (16a). Red solid (307 mg, 47% yield); mp >250 °C (EtOH 95%); R_f = 0.59 (CH_2Cl_2/MeOH 9:1); IR 1645, 3314 cm^{-1}; ^{1}H NMR ($CDCl_3$, TFA-d_1) δ 3.70−3.75 (m, 4H), 4.05 (s, 3H), 4.13 (s, 3H), 4.17 (s, 2H), 7.66 (s, 1H), 7.65−7.78 (m, 3H), 8.46 (s, 1H), 11.07 (bs, 2H); MS (EI) m/z 318 (M^+, 65), 303 (100). Anal. ($C_{20}H_{18}N_2O_2$·HCl·1.5H_2O) C, H, N.

10-Hydroxy-4,5-dimethoxy-1,2,3,12-tetrahydroindeno-[1,2-*b*]pyrido[4,3,2-*de*]quinoline Hydrochloride (16b). Orange solid (235 mg, 53% yield); mp >250 °C (MeOH/Et_2O/H_2O); R_f = 0.49 (CH_2Cl_2/MeOH 9:1); IR 1649, 3396 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.23−3.46 (m, 4H), 3.75 (s, 3H), 3.95 (s, 3H), 4.00 (s, 2H), 6.72 (bs, 2H), 7.00 (d, J = 8.3, 1H), 7.06 (s, 1H), 7.13 (s, 1H), 8.44 (d, J = 8.3, 1H), 10.71 (s, 1H); MS (EI) m/z 334 (M^+, 58), 319 (100). Anal. ($C_{20}H_{18}N_2O_3$·HCl·2H_2O) C, H, N, Cl.

4,5,10-Trimethoxy-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Hydrochloride (16c). Red solid (276 mg, 62% yield); mp >250 °C (EtOH 95%); R_f = 0.65 (CH_2Cl_2/MeOH 9:1); IR 1650, 3321 cm^{-1}; ^{1}H NMR ($CDCl_3$, TFA-d_1) δ 3.67 (bt, 2H), 3.98 (s, 3H), 4.03 (s, 4H), 4.10 (s, 3H), 4.15 (s, 3H), 7.18 (d, J = 8.5, 1H), 7.21 (s, 1H), 7.72 (s, 1H), 8.36 (d, J = 8.5, 1H); MS (EI) m/z 348 (M^+, 61), 333 (100). Anal. ($C_{21}H_{20}N_2O_3$·HCl·0.2H_2O) C, H, N.

4,5-Dimethoxy-10-[3-(4-methyl)piperazin-1-ylpropoxy]-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Tetrahydrochloride (16d). Orange solid (193 mg, 42% yield); mp >250 °C (EtOH/Et_2O); R_f = 0.06 (CH_2Cl_2/MeOH 9:1, NH_3); IR 1649, 3143 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.02 (p, J = 5.4, 2H), 2.63−3.59 (m, 17H), 3.83 (s, 3H), 4.03 (s, 3H), 4.08 (s, 2H), 4.12 (t, J = 5.4, 2H), 7.16 (d, J = 8.7, 1H), 7.30 (s, 1H), 7.59 (s, 1H), 8.41 (d, J = 8.7, 1H); MS (EI) m/z 474 (M^+, 100), 404 (68), 334 (36), 141 (78). Anal. ($C_{28}H_{34}N_4O_3$·4HCl·2H_2O) C, H, N, Cl.

4,5-Dimethoxy-12-methyl-1,2,3,12-tetrahydroindeno-[1,2-*b*]pyrido[4,3,2-*de*]quinoline Hydrochloride (16g). Light-red solid (233 mg, 52% yield); mp >250 °C (EtOH 95%); R_f = 0.57 (CH_2Cl_2/MeOH 9:1); IR 1650, 3442 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.55 (d, J = 7.1, 3H), 3.38−3.50 (m, 2H), 3.60−3.75 (m, 2H), 3.89 (s, 3H), 4.07 (s, 3H), 4.29 (q, J = 7.5, 1H), 5.11 (bs, 2H), 7.52 (s, 3H), 7.83 (s, 1H), 9.12 (s, 1H); MS (EI) m/z 332 (M^+, 70), 317 (100). Anal. ($C_{21}H_{20}N_2O_2$·HCl·H_2O) C, H, N.

4,5-Dimethoxy-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido-[4,3,2-*de*]quinolin-12-one Hydrochloride (16h). Red solid (269 mg, 60% yield); mp 250 °C (EtOH 95%); R_f = 0.89 (CH_2Cl_2/MeOH 9:1); IR 1650, 1709, 3407 cm^{-1}; ^{1}H NMR ($CDCl_3$, TFA-d_1) δ 3.73 (t, J = 6.1, 2H), 3.83 (t, J = 6.1, 2H), 4.00 (s, 3H), 4.12 (s, 3H), 7.47 (s, 1H), 7.73−8.19 (m, 3H), 8.54 (d, J = 7.6, 1H), 10.34 (bs, 2H); MS (EI) m/z 332 (M^+, 75), 317 (100). Anal. ($C_{20}H_{16}N_2O_3$·HCl·H_2O) C, H, N.

10-(2-Aminoethoxy)-4,5-dimethoxy-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Dihydrochloride (16j). Red solid (233 mg, 50% yield); mp >250 °C (MeOH/Et_2O); R_f = 0.11 (CH_2Cl_2/MeOH 9:1, NH_3); IR 1642, 3419 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.26−3.28 (m, 4H), 3.46 (t, J = 6.5, 2H), 3.75 (s, 3H), 3.98 (s, 3H), 4.09 (s, 2H), 4.33 (t, J = 4.7, 2H), 6.95 (s, 1H), 7.25 (d, J = 8.8, 1H), 7.42 (s, 1H), 8.40 (d, J = 8.5, 1H); MS (EI) m/z 377 (M^+, 96), 362 (64), 319 (100). Anal. ($C_{22}H_{23}N_3O_3$·2HCl·3H_2O) C, H, N, Cl.

10-(3-Aminopropoxy)-4,5-dimethoxy-1,2,3,12-tetrahydroindeno[1,2-*b*]pyrido[4,3,2-*de*]quinoline Dihydrochloride (16k). Red solid (253 mg, 56% yield); mp >250 °C (MeOH/Et_2O); R_f = 0.08 (CH_2Cl_2/MeOH 9:1, NH_3); IR 1644, 3419 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.08 (p, 2H), 2.98 (t, J = 6.8, 2H), 3.26 (t, 2H), 3.47 (t, 2H), 3.74 (s, 3H), 3.97 (s, 3H), 4.06 (s, 2H), 4.19 (t, J = 4.7, 2H), 6.92 (s, 1H), 7.17 (d, J = 8.8, 1H), 7.34 (s, 1H), 8.33 (d, J = 8.5, 1H); MS (EI) m/z 391 (M^+, 100), 376 (84), 319 (100), 243 (88). Anal. ($C_{23}H_{25}N_3O_3$·2HCl·2H_2O) C, H, N, Cl.

Biological Methods. Melting Temperature Measurements. 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 path length) were heated by circulating water. Measurements were performed using 20 μM poly(dAT)$_2$ 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 absorption spectra.

Fluorescence Measurements. Binding studies were carried out through a competitive displacement fluorometric assay with DNA-bound ethidium.[18,19] Fluorescence data were recorded at room temperature with a SPEX Fluorolog fluorometer. Excitation was set at 515 nm, and fluorescence emission was monitored over the range 550−700 nm. Experiments were performed with an [ethidium]/[DNA] molar ratio of 12.6:10 and a drug concentration range of 0.01−100 μM in a BPE buffer, pH 7.1. C_{50} values for ethidium displacement were calculated using a fitting function incorporated into Prism 3.0, and the apparent equilibrium binding constants (K_{app}) were calculated as follows: $K_{app} = (1.26\ \mu M/C_{50})K_{ethidium}$, with $K_{ethidium} = 10^7$ M^{-1}.

Circular Dichroism. The CD spectra were obtained with a J-810 Jasco spectropolarimeter at 20 °C controlled by a PTC-424S/L Peltier type cell changer (Jasco). A quartz cell of 10 mm path length was used to obtain spectra from 500 to 230 nm with a resolution of 0.1 nm. The desired ratios of compound to DNA were obtained by adding DNA to the cell containing a constant amount of the drug (20 μM). The CD measurements were performed in a 1 mM Na cacodylate, buffer pH 7.0.

Electric Linear Dichroism. ELD measurements were performed with a computerized optical measurement system using the previously outlined procedures.[20,21] All experiments were conducted with a 10 mm path length Kerr cell having a 1.5 mm electrode separation. The samples were guided under an electric field strength varying from 1 to 14 kV/cm. The drug under test was present at 10 μM together with the DNA at 250 μM unless otherwise stated. The ELD measurements were performed in a 1 mM Na cacodylate buffer, pH 7.0.

DNase I Footprinting. The experimental procedure has been previously described.[22,23]

Cell Culture and Growth Assays. Human prostate cancer PC 3 cells were maintained in RPMI 1640 culture medium supplemented with 10% FCS. For growth assays, the cells were seeded onto 96-well plates at a density of 3×10^4 cells/well. After 3 days, the cell medium was changed to serum-free medium and the cells were starved for 24 h for culture synchronization. The stimulation of the growth of quiescent cells was then performed by 10 ng/mL EGF plus TSe (50 ng/mL transferrin and 50 pg/mL selenium), and the tested compounds were added to the culture medium. After an additional 72 h, cell growth was estimated by the colorimetric MTT test.[24]

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Supporting Information Available: Elemental analyses for compounds **9i**, **14a**−**d**,**g**−**k**, **15d**,**j**,**k**, and **16a**−**d**,**g**,**h**,**j**,**k**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Thurston, D. E. Nucleic acid targeting: therapeutic strategies for the 21st century. *Br. J. Cancer* **1999**, *80*, 65−85.
(2) Hurley, L. H. DNA and its associated processes as targets for cancer therapy. *Nat. Rev. Cancer* **2002**, *2*, 188−200.
(3) Charmantray, F.; Demeunynck, M.; Carrez, D.; Croisy, A.; Lansiaux, A.; Bailly, C.; Colson, P. 4-Hydroxymethyl-3-aminoacridine derivatives as a new family of anticancer agents. *J. Med. Chem.* **2003**, *46*, 967−977.
(4) Pindur, U.; Kim, Y.-S.; Mehrabani, F. Advances in indolo[2,3-*a*]carbazole chemistry: Design synthesis of protein kinase C and topoisomerase I inhibitors. *Curr. Med. Chem.* **1999**, *6*, 29−69.
(5) Braña, M. F.; Cacho, M.; Gradillas, A.; de Pascual-Teresa, B.; Ramos, A. Intercalators as anticancer drugs. *Curr. Pharm. Des.* **2001**, *7*, 1745−1780.
(6) Doan Thi Mai, H.; Gaslonde, T.; Michel, S.; Tillequin, F.; Koch, M.; Bongui, J.-B.; Elomri, A.; Seguin, E.; Pfeiffer, B.; Renard, P.; David-Cordonnier, M.-H.; Tardy, C.; Laine, W.; Bailly, C.; Kraus-Berthier, L.; Léonce, S.; Hickman, J.-A.; Pierré, A. Structure−activity relationships and mechanism of action of antitumor benzo[3,2-*h*]acridin-7-one analogs of acronycine analogues. *J. Med. Chem.* **2003**, *46*, 3072−3082.
(7) Gregson, S. J.; Howard, P. W.; Hartley, J. A.; Brooks, N. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. Design, synthesis, and evaluation of a novel pyrrolobenzodiazepine DNA-interactive agent with highly efficient cross-linking ability and potent cytotoxicity. *J. Med. Chem.* **2001**, *44*, 737−748.
(8) Cozzi, P. A new class of cytotoxic DNA minor groove binders: alpha-halogenoacrylic derivatives of pyrrolecarbamoyl oligomers. *Farmaco* **2001**, *56*, 57−65.
(9) Dervan, P. B. Molecular recognition of DNA by small molecules. *Bioorg. Med. Chem.* **2001**, *9*, 2215−2235.
(10) Chakal, S.; Facompré, M.; Houssin, R.; Goossens, J.-F.; Pommery, N.; Hénichart, J.-P.; Bailly, C. Highly cytotoxic benzo[*c*]-pyrido[2,3,4-*kl*]acridines. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 943−946.
(11) Pilch, D. S.; Yu, C.; Makhey, D.; LaVoie, E. J.; Srinivasan, A. R.; Olson, W. K.; Sauers, R. R.; Breslauer, K. J.; Geacintov, N. E.; Liu, L. F. Minor groove-directed and intercalative ligand−DNA interactions in the poisoning of human DNA topoisomerase I by protoberberine analogs. *Biochemistry* **1997**, *36*, 12542−12553.
(12) Dassonneville, L.; Wattez, N.; Baldeyrou, B.; Mahieu, C.; Lansiaux, A.; Banaigs, B.; Bonnard, I.; Bailly, C. Inhibition of topoisomerase II by the marine alkaloid ascididemin and induction of apoptosis in leukemia cells. *Biochem. Pharmacol.* **2000**, *60*, 527−537.
(13) Chackal, S.; Houssin, R.; Hénichart, J.-P. An efficient synthesis of the new benzo[*c*]pyrido[2,3,4-*kl*]acridine skeleton. *J. Org. Chem.* **2002**, *67*, 3502−3505.
(14) Cheng, C. C.; Yan, S. J. The Friedländer synthesis of quinolines. In *Organic Reactions*; Dauben, W. G., Ed.; John Wiley: New York, 1982; Vol. 28, pp 37−201.
(15) 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) Katchalski, E.; Ishai, D. B. 2-Oxazolidones: synthesis from *N*-carbalkoxy-*β*-haloalkylamines. *J. Org. Chem.* **1950**, *15*, 1067−1073.
(17) Schneider, F.; Bernauer, K.; Guggisberg, A.; van den Broek, P.; Hesse, M.; Schmid, H. Synthesis of (+)-oncinotine. *Helv. Chim. Acta* **1974**, *57*, 434−440.
(18) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. Potential antitumor agents. 34. Quantitative relationships between DNA binding and molecular structure for 9-anilinoacridines substituted in the anilino ring. *J. Med. Chem.* **1981**, *24*, 170−177.
(19) Pavlov, V.; Kong Thoo Lin, P.; Rodilla, V. Cytotoxicity, DNA binding and localisation of novel bis-naphtilimidopropylamine derivatives. *Chem.-Biol. Interact.* **2001**, *137*, 15−24.
(20) 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 Publishing Corporation: New York, 1981; pp 363−398.
(21) Houssier, C.; O'Konski, C. T. Electro-optical instrumentation systems with their data acquisition and treatment. In *Molecular Electro-Optics*; Krause, S., Ed.; Plenum Publishing Corporation: New York, 1981; pp 309−339.
(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.
(23) Bailly, C.; Kluza, J.; Ellis, T.; Waring, M. J. DNase I footprinting of small molecule binding sites on DNA. In *DNA Synthesis: Methods and Protocols. Methods in Molecular Biology*; Herdewijn, P., Ed.; Humana Press, in press.
(24) Hirst, S. J.; Barnes, P. J.; Twort, C. H. C. Quantifying proliferation of cultured human and rabbit airway smooth muscle cells in response to serum and platelet-derived growth factor. *Am. J. Respir. Cell Mol. Biol.* **1992**, *7*, 574−581.

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