Benzo[*f*]azino[2,1-*a*]phthalazinium Cations: Novel DNA Intercalating Chromophores with Antiproliferative Activity

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New azaquinolizinium-type cations have been obtained from isochromane. The synthesis was completed over seven steps and included as the key feature an intramolecular Westphal condensation. This first example of the intramolecular process allowed the preparation of benzo[*f*]pyrido[2,1-*a*]phthalazinium and benzo[*f*]quino[2,1-*a*]phthalazinium salts, which were evaluated as DNA intercalators, DNA topoisomerase I inhibitors, and antiproliferative compounds. Both cationic systems behave as DNA intercalators and exhibit antiproliferative activity. The pentacyclic benzo[*f*]quino[2,1-*a*]phthalazinium cations also have an inhibitory effect on the catalytic activity of DNA topoisomerase I, without trapping of cleavage complexes. Structural characterization using density functional theory indicates that the fused ring systems are slightly nonplanar, and additional molecular modeling studies suggest a preferred orientation for the intercalating chromophores within a typical CpG or TpG intercalation site.

Introduction

Many effective anticancer drugs in clinical use interact with DNA,[1−4] and some of them do so through intercalation.[5,6] The intercalation process reflects the ability of a planar aromatic or heteroaromatic system to become inserted between adjacent base pairs of a DNA molecule without disturbing the overall stacking pattern.[7] This class of DNA-binding ligands can affect many biological properties of DNA, including replication and transcription, and several antitumor antibiotics are recognized as the most important class of DNA intercalating reagents.[8,9]

The intercalative reaction is envisaged as a complex process that depends on multiple variables, with microheterogeneity of the DNA molecule being one of the factors that more strongly contributes to hamper our understanding of the rules that govern high-affinity binding and selection of a particular sequence.[10] Thus, in this field there is still room for the design and exploration of new structural classes of chromophores with intercalating properties[11−16] or for rational structural modifications on well-established chromophores, even though we must assume that understanding the mechanisms that ultimately lead to cell death is still an elusive goal.

We recently reported the promising antiproliferative activity associated with a new class of DNA intercalators based on an azaquinolizinium core, such as γ-carbolinium (**1**)[17,18] and benzimidazolium (**2**)[19,20] systems (Figure 1). Results from our lab are demonstrating that, contrary to other charged DNA intercalators, the requisite for intercalation in this class of hetearoaromatic cations bearing a bridgehead quaternary nitrogen is the existence of at least four fused rings, preferably nonlinearly fused. Moreover, while affinity seems to be little affected by simple substituents, the antiproliferative activity is strongly dependent on chromophore substitution.[17−21]

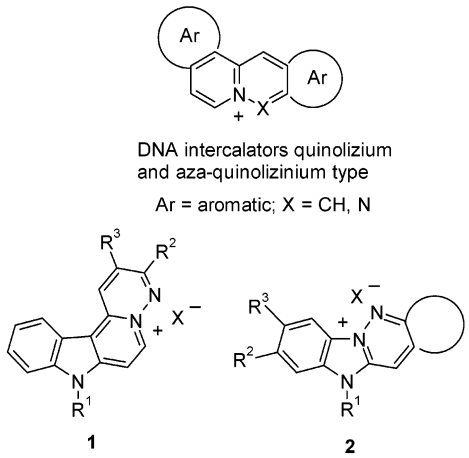

Figure 1. General structure and representative examples (**1**, **2**) of azaquinolizinium-type DNA intercalators.

These considerations prompted us to explore a new series of azaquinolizinium-type chromophores with potential DNA intercalating properties. Herein we report the synthesis and DNA binding affinity of one of these fused heteroaromatic cationic systems by using the intramolecular Westphal reaction, a version of the intermolecular process[22] widely used in the synthesis of quinolizinium and azaquinolizinium cations.

Chemistry

In earlier reports we established the potential of the Westphal condensation reaction (Scheme 1) to prepare

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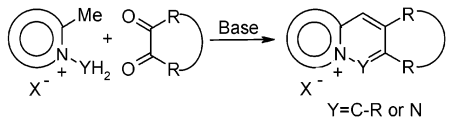
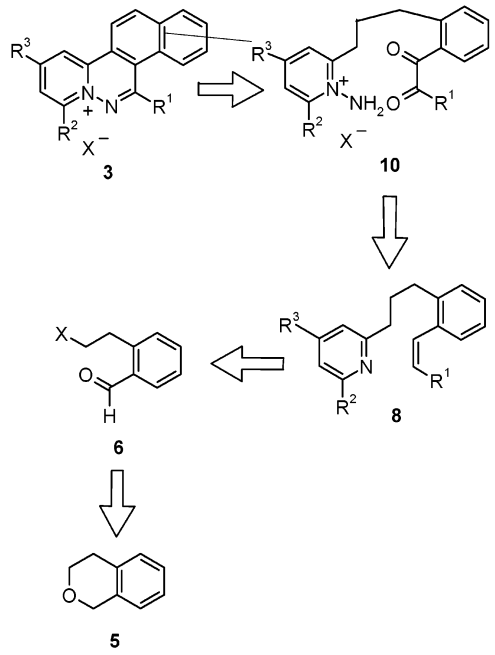
Scheme 1



Scheme 2



a great variety of heteroaromatic cations bearing a quaternary bridgehead nitrogen, some of which behave as DNA intercalators.[17−22]

The search in our lab for new azaquinolizinium-type cations having the requisites previously established for intercalation led us to focus on different possibilities for incorporating the azaquinolizinium moiety into a nonlinearly fused tetracyclic cation. The retrosynthetic analysis of one of the desired systems (Scheme 2) indicated that the intramolecular Westphal reaction[23] could be the choice for a straightforward synthesis of benzo[*f*]pyrido[2,1-*a*]phthalazinium cations **3** from the salt **10**, which was envisioned to originate from the alkene **8**. Thus, our approach was to use isocromane **5** as the key raw material, convert it to a 2-haloethyl benzaldehyde **6**, and prepare **8** through a Wittig reaction.

Formation of the 2-bromoethylbenzaldehyde **6** (X = Br) from **5** was carried out following the procedure described by Rieche.[24] The method rests on the treatment of **5** with bromine in CH_2Cl_2, at reflux temperature under sunlight. The Wittig reaction of **6** with alkyl and arylmethylphosphines in THF gave rise to a 1:1 *Z*/*E* mixture of the required alkenes **7** in moderate to good yields. The success of the reaction required slow addition of the bromo derivative to avoid undesirable competitive dehydrohalogenation.

Reaction of **7** with methylides generated from picoline and lutidine in the presence of *n*BuLi in THF afforded the corresponding derivatives **8** in good yield, except when **7a** reacted with the 2,4-dimethylpyridine, where the desired derivative **8c** was obtained in only 37% yield because of the formation of the undesired regioisomer (34% yield) coming from the reaction of **7a** with 4-picolinmethylide. Carrying out the nucleophilic substitution under different conditions did not improve the regioselectivity.

Completion of the synthesis of **3** would require the following synthetic operations: (1) the preparation of the diketones **9**, (2) formation of the salts **10**, and finally (3) intramolecular Westphal reaction cyclization of the *N*-aminopyridinium salts under basic conditions.

The most successful methods for conversion of alkenes into diketones are based on the use of DMSO. More specifically, good yields in diketone are described by reaction of arylalkenes and I_2/DMSO or HBr/DMSO. In our case, compounds **8a**−**e** (R^1 = Ar) were oxidized to the corresponding diketones in moderate yields by using either iodine[25,26] or HBr[27] in DMSO at reflux temperature, although in a comparative study with compounds **8a,b** the latter procedure gave better yields (**9a**, 77% vs 60%; **9b**, 71% vs 56%). All our attempts to transform the alkene with R^1 = Me into the corresponding diketones failed with oxidative degradation of the double bond and concomitant formation of aldehydes being observed.

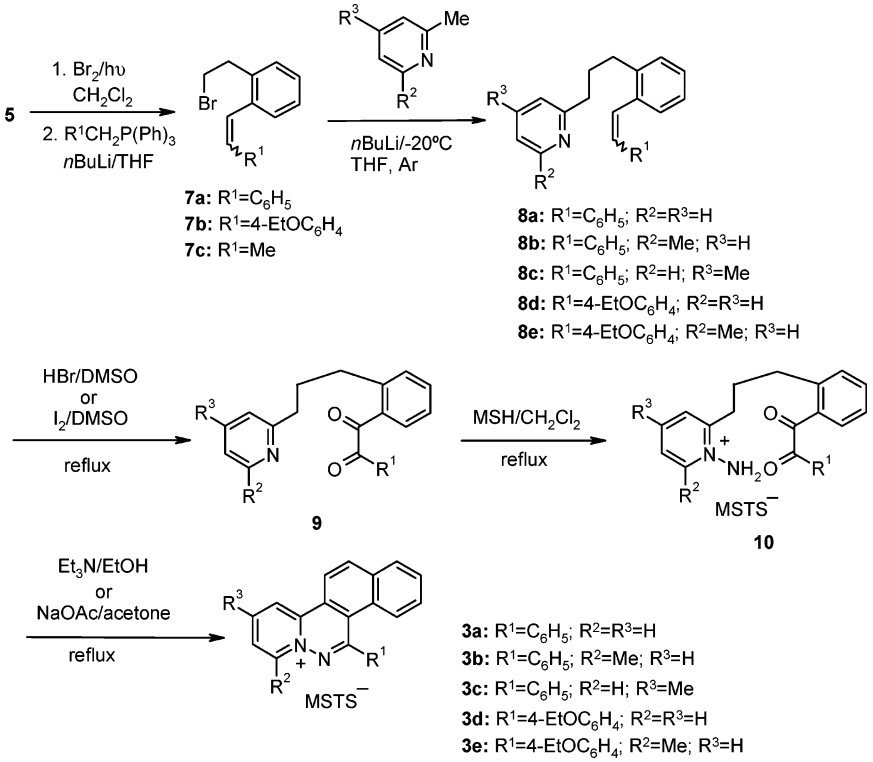
Compounds **9** were easily transformed into the corresponding *N*-aminopyridinium salts **10** by reaction with (*O*-mesitylenesulfonyl)hydroxylamine (MSH) in CH_2Cl_2 at reflux temperature in good yields. Initial experiments on the intramolecular Westphal condensation focused on the selection of the most appropriate base and solvent for the success of the reaction. On the basis of our previous work on the intermolecular process,[22] the condensation was achieved using triethylamine in ethanol and sodium acetate in acetone. In most of the cases, the reaction afforded a complex mixture from which the expected condensation product and the required fully oxidized compound **3** were identified. Although both compounds were difficult to separate, it was found that prolonged reaction times gave **3** as the only compound without appreciable loss of yield.

To prove the DNA intercalating properties of compounds **3**, both electrophoresis and UV determination (for details, see below) were carried out. It was shown that compound **3c** does not interact with DNA and that compounds **3a**, **3b**, and **3e** intercalate into DNA only weakly. The solubility of compound **3d** was too low to allow an accurate measurement of its intercalating behavior. In the light of these findings, we decided to modify the size of the chromophore, thereby extending the fused system with an additional aromatic ring.

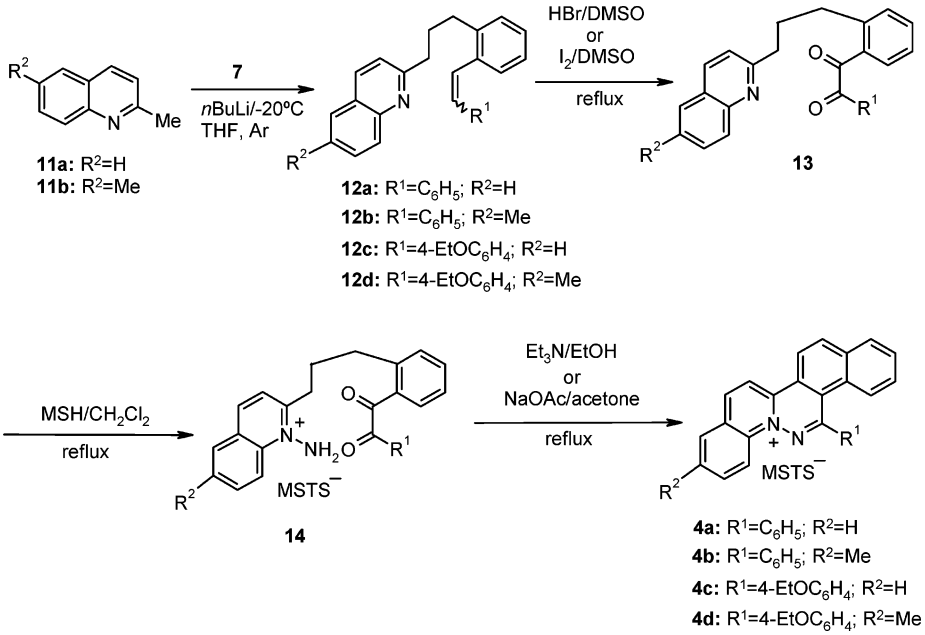
To use the same approach for the preparation of the new chromophore, 2-methylquinoline, 3-methylisoquinoline, and 2-methylbenzothiazole were chosen as heterocycles for the reaction with **7**. However, only the use of 2-methylquinoline allowed us to complete the synthetic route leading to the pentacyclic benzo[*f*]quino[2,1-*a*]phthalazinium system **4** (Scheme 4). The reaction with 3-methylisoquinoline failed under different conditions with the heterocycle being recovered unaltered. On the contrary, 3-methylbenzothiazole reacted with **7a**, affording the substitution compound. However, the subsequent oxidation step was also troublesome and complex reaction mixtures were formed under the tested conditions.

In this case an alternative method for the formation of diketones **13** with substituent R^1 being an alkyl group

Scheme 3



Scheme 4



was also attempted using the Katritzky benzotriazole methodology,[28] but up to now all our attempts have been unsuccessful.

Table 1 shows the heteroaromatic cations **3** and **4** prepared along with the best conditions for the intramolecular Westphal reaction and the yields obtained in this last key step of the synthesis.

Results and Discussion

DNA Binding Studies. As indicated above, two different studies were carried out to investigate the DNA-binding behavior of the benzo[*f*]pyrido[2,1-*a*]phthalazinium salts **3** and benzo[*f*]quino[2,1-*a*]phthalazinium salts **4**. Supporting evidence for an intercalative mode of binding to DNA of compounds **3a**,**b**,**e** and **4a**−**d** was first obtained by electrophoretic titration of a bacterial plasmid, as shown in Figure 2. This assay exploits the conformational changes induced by intercalating ligands on covalently closed, double-stranded DNA molecules, such as bacterial plasmids, together with their differential electrophoretic behavior in agarose gels.

Bacterial plasmids are dynamically maintained in vivo in an underwound topology that induces a negatively supercoiled conformation. Upon binding of a number of intercalating molecules of any kind, the

Table 1. Synthesis and DNA Binding Properties of Benzo[*f*]pyrido[2,1-*a*]phthalazinium Salts **3** and Benzo[*f*]quino[2,1-*a*]phthalazinium Salts **4**[a]

compd	R^1	R^2	R^3	reaction conditions	yield (%)	$\Delta\lambda$	H	K	n
3a	C_6H_5	H	H	A	65	10	6	ND	ND
3b	C_6H_5	Me	H	B	50	0	0	–	–
3c	C_6H_5	H	Me	B	28	0	0	–	–
3d	4-EtO-C_6H_4	H	H	A	22	–	–	NM	NM
3e	4-EtO-C_6H_4	Me	H	B	28	0	0	–	–
4a	C_6H_5	H	–	A	75	12	33	2.3	2.3
4b	C_6H_5	Me	–	B	50	10	11	1.03	1.8
4c	4-EtO-C_6H_4	H	–	B	28	12	52	7.20	2.3
4d	4-EtO-C_6H_4	Me	–	B	50	14	6	1.12	2.2
EtBr								2.06	2.1

[a] Yields refer to the intramolecular Westphal reaction step. A: NaOAc/acetone. B: Et_3N/EtOH. $\Delta\lambda = (\lambda_{bound} - \lambda_{free})$, where λ_{free} and λ_{bound} are the wavelengths of maximum absorption for free and DNA bound compounds. H = percent hypochromicity [$\%H = (1 - \epsilon_{bound}/\epsilon_{free}) \times 100$], where ϵ_{free} and ϵ_{bound} are the extinction coefficients for free and DNA bound compounds. K = the affinity constant for DNA (10^5 M^{-1}). n = the number of base pairs per bound molecule. EtBr =ethidium bromide. ND = not determined. NM = not measurable due to the insolubility of the compound above the micromolar range.

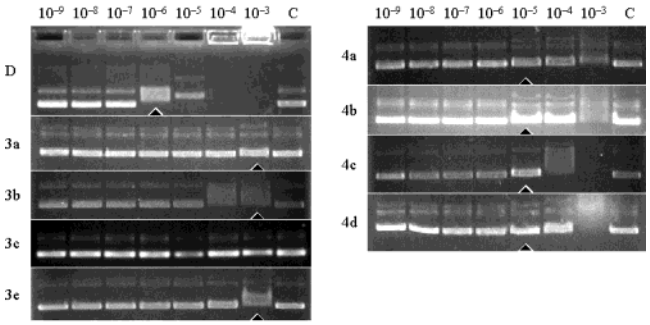


Figure 2. Electrophoretic titration of supercoiled bacterial plasmid DNA. Each lane was loaded with a 6 μL sample containing a preincubated mixture of 200 ng of plasmid DNA plus the compound under study at the final molar concentration indicated in the top row. Pictures were taken after postelectrophoretic staining with ethidium bromide. The arrowhead in each gel indicates the lane where a complete relaxation of the plasmid DNA has been reached or exceeded. The effect was confirmed in all cases by a second assay exploring a narrower range of concentrations. The left panel shows the results obtained with compounds **3** (**3d** could not be analyzed because of its very low solubility in aqueous media). The same assay applied to ditercalinium (D) is also shown for comparison. The right panel shows the results obtained with compounds **4**.

plasmids reach a relaxed conformation. The binding of more intercalating molecules then drives the plasmids to adopt a positively supercoiled form. Such complex conformational changes can be detected by viscometry as well as by agarose gel electrophoresis. Nevertheless, the electrophoretic assay, described in the Experimental Section, is much faster and requires very small amounts of both plasmid DNA and ligand. We use this electrophoretic assay as a first screening test to determine the intercalative capacity of every new molecule. Both positive and negative supercoiled conformations behave as compact structures, with a fast electrophoretic migration in agarose gel, whereas the more extended and flexible relaxed conformation has a much slower electrophoretic migration. The results obtained by this assay with compounds **3** and **4** are shown in Figure 2. The complete conformational transition is clearly visible in the case of intercalating compounds that form highly stable complexes with DNA, that is, when the kinetic dissociation constant (k_{off}) of the binding reaction is very low. Such slow dissociation behavior is usually found in the case of bis-intercalating compounds. Ditercalinium[29] was included in Figure 1 to show an example of the typical results obtained with a bisintercalating molecule, for which this assay was initially designed. In the case of monointercalating molecules, the k_{off} is usually much higher, so that dissociation occurs very rapidly when the DNA:ligand complexes enter the agarose gel. In this case, the relaxed conformation occurs only as a transient state. All of the plasmid DNA can be considered free of the intercalating ligand after a few minutes of electrophoresis and, in this situation, the plasmids recover the fast migrating, negatively supercoiled conformation. Nevertheless, in some lanes the plasmids start from, or pass through, the relaxed state inside the gel, and this conformation has a much slower electrophoretic migration than the negative or positive supercoiled states. The resulting effect is a transient reduction in the migration speed of the DNA band that is revealed by its slight delay when compared to a control lane, or by a smearing of the DNA band.

Compounds with high kinetic dissociation constants (k_{off}) or with an overall reduced intercalative binding capacity may produce negative or somewhat unclear results in this preliminary, but semiquantitative, screening assay. Ethidium is a noteworthy example of a well-known DNA-intercalating compound that produces a negative result in this assay, due to the high dissociation rate of its complex with DNA. For this reason, a further indication of the intercalative binding capacity can be obtained by a different electrophoretic assay, which is much more sensitive as a DNA intercalation test, but only qualitative and also more demanding in terms of labor, reagents, and expense of analyte. In this type of experiment, the compound under study is included in the mass of the agarose gel, as well as in the samples, to maintain the plasmids in a saturating environment during the electrophoresis. The complete assay involves four lanes. In two of them the plasmid is previously treated with DNA topoisomerase I in order to obtain a covalently closed double-stranded DNA in a relaxed topology. The other two lanes are loaded with the original plasmids, as purified from bacterial culture in a natural negatively supercoiled topology. If intercalation occurs, all the plasmids in the four lanes should adopt a positively supercoiled conformation, characterized by a likewise fast electrophoretic migration. If the compound under study is not able to intercalate at all, the lanes loaded with the topoisomerase I-relaxed plasmids show the characteristic slow migrating band, clearly distinguishable from the fast migrating band that appears in the lanes loaded with the negatively supercoiled plasmids. In two of the four lanes, the putative ligand is not included in the sample. These two lanes serve as an internal control to ascertain that conditions inside the gel are saturating as expected. As an additional control, the same set of samples is also applied to a second agarose gel without the compound under study. This assay complements the previous one, mainly to resolve some unclear or conspicuous situations as in the noteworthy case of compound **3c**, for which a

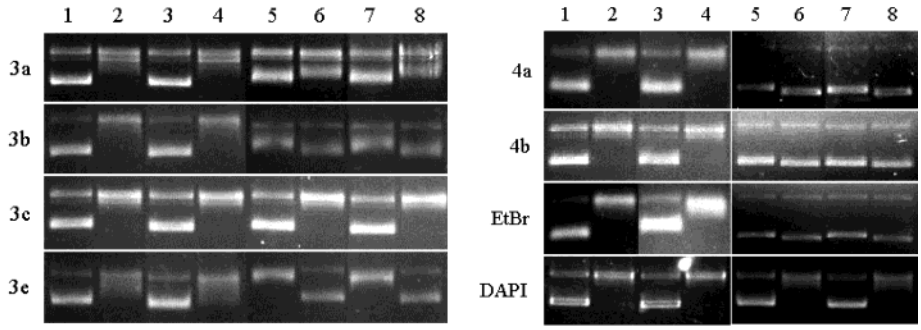


Figure 3. Electrophoretic assay of supercoiled and relaxed bacterial plasmid DNA. Each lane was loaded with 6 μL samples containing 200 ng of bacterial plasmid DNA that was untreated, negatively supercoiled in the case of odd numbered lanes, or relaxed by treatment with DNA topoisomerase I in the case of even numbered lanes. The samples applied to lanes 3, 4, 7, and 8 contain the compound indicated on the left at a final concentration of 0.1 mM. The agarose gel on the right-hand side of each row contains the same compound in the mass of the gel at a final concentration of 0.01 mM. Pictures were taken after postelectrophoretic staining with ethidium bromide. The left panel shows the results obtained with compounds **3** (**3d** could not be analyzed because of its complete insolubility in aqueous media). The right panel shows the results obtained with compounds **4a** and **4b**. The results of the same assay when applied to ethidium bromide (EtBr) and DAPI are also shown to compare the behavior of a well-known intercalator and a groove binder, respectively.

simple substituent completely abolishes the intercalating capacity of the molecule. The results obtained from this assay are shown in Figure 3.

Compounds **4a**−**d** show a similar intercalative binding behavior in these two electrophoretic assays. In the titration assay the fully relaxed conformation occurs at concentrations around 10^{-5} M in the four cases (intercalator:base-pair molar ratio of 0.195). Compounds **3a**, **3b**, and **3e** also display intercalating properties. In these cases the fully relaxed conformation is reached at 10^{-3} M (intercalator:base-pair molar ratio of 19.53). Interestingly, when a methyl substituent is introduced on the parent chromophore, the resulting compound **3c** does not intercalate at all (see Figures 2 and 3).

UV−visible spectrophotometric determinations of series **3** and **4** showed that compounds **3b**, **3c**, and **3e** exhibit neither hypochromic nor bathochromic effects on their UV spectra in the presence of DNA, thus confirming the very weak interaction of these compounds with DNA. Compound **3a** and the pentacyclic salts **4a**−**d** showed characteristic changes in their visible absorption spectra as a result of formation of more stable complexes. Both a hypochromic effect (percent of hypochromicity between 6 and 52%) and bathochromic shifts between 10 and 14 nm relative to the spectra of the free compounds[30,31] were observed (Table 1).

Composite spectra exhibit well-defined isosbestic points for this series (one single binding mode) (Figure 4). From the spectral changes, nonlinear Scatchard binding isotherms (Figure 5) were generated for the interaction of these compounds with DNA using the noncooperative McGhee−Von Hippel equation.[32]

The binding parameters (K, n) determined in this manner are shown in Table 1, with K being the apparent binding constant and n the number of base pairs per bound molecule. For compound **3a** the numerical data generated in different spectrophotometric titrations could not be adequately fitted to the Scatchard equation because of its dispersion. All derivatives **4a**−**d** have DNA affinity constant values around 10^5 M^{-1}, which are essentially on the same order of magnitude as that of the well-known intercalator ethidium bromide,[33−35] used here as a reference and measured under the same conditions (Table 1).

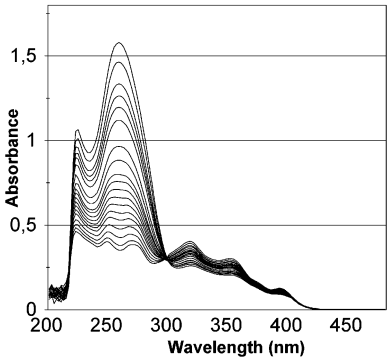


Figure 4. Binding curves for **4a** to calf thymus DNA showing the isosbestic point. Spectrophotometric titration was performed by serial addition of 100 μL aliquots of a DNA solution (47×10^{-6} M) into a 10 cm path length quartz cell containing a solution of **4a** (1.5×10^{-6} M) and scanning the UV−visible spectrum after each addition.

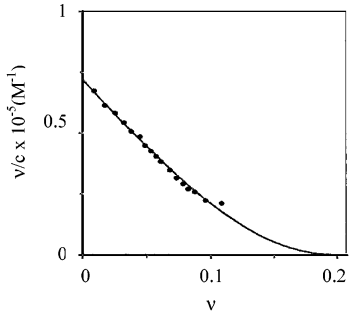


Figure 5. Scatchard plot for the binding of **4a** to calf thymus DNA. Values for binding ratio v and free compound concentration c were determined from data taken from spectrophotometric titrations of the compound by DNA. Points in the figure are the data, and solid lines are the best fits from the noncooperative McGhee−von Hippel model.[32]

Theoretical Calculations. Representative compounds **3a** and **4a** were studied using density functional theory with the well-established B3LYP/6-31G* method. The presence of the phenyl substituent induces some distortions that cause the fused ring system to which it is attached to be slightly nonplanar (Figure 6). This phenyl ring is locked into position as rotation is hampered by neighboring atoms. Similar distortions due to steric congestion have been recently reported for related phthalocyanines.[36]

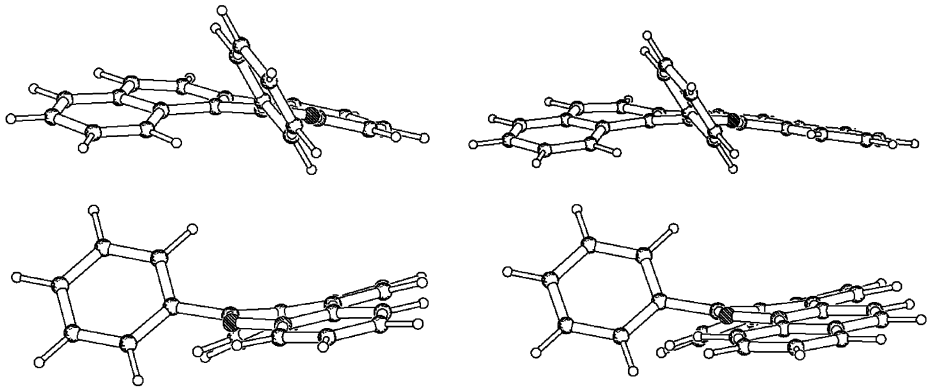


Figure 6. Front and side views of representative compounds **3a** (left) and **4a** (right) optimized using the density functional B3LYP/6-31G* method.

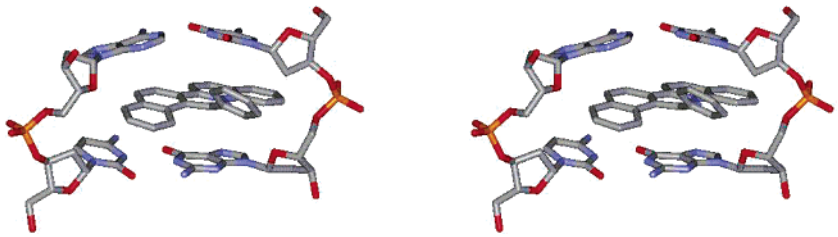


Figure 7. Stereoview from the minor groove of compound **4a** docked into a TpG intercalation step (for clarity, only non-H atoms are displayed).

When the complexes between **3a** and **4a**−**d** with prototypical CpG and TpG intercalation steps were simulated using molecular dynamics, only the complexes in which the phenyl ring substituent protruded from the minor groove led to stable trajectories (Figure 7). The alternative orientation of the chromophore with the phenyl pointing out from the major groove resulted, in all cases, in extrusion of the ligand out of the intercalation step. We take these results as an indication that the intercalation mode of these molecules is similar to that of ethidium bromide.[10,33−35]

DNA Topoisomerase I inhibition. This enzyme is a well-known biological target for some potent anticancer drugs, which exert their actions due a particular mechanism of inhibition that involves trapping the covalent enzyme−DNA "cleavage" complex.[37−40] The inhibitory effects on the catalytic activity of Topo1 were semiquantitatively determined[41] for all synthesized compounds, and the results (not shown) indicate that compounds **4a**, **4b**, and **4d** have inhibitory effects on Topo1. Compound **4d** inhibits this enzyme completely at a concentration of 10 μM, and partially at 5 μM, whereas compounds **4a** and **4b** show only partial inhibition at 10 μM. In all cases the mechanism of the inhibition does not involve trapping of the cleavage complex. This fact very likely rules out this particular enzyme as the molecular target responsible for the cytotoxic effects of these compounds, but the inhibition does not seem to result simply from nonspecific steric interference, since the inhibitory effect is detectable at ligand concentrations well below saturation of the DNA substrate. Compounds **3a**−**c**, **3e**, and **4c** do not show any effect on the enzymatic activity of Topo1.

Antiproliferative Activity. In vitro activity potencies (GI_{50}) of compounds **3** and **4** against different human cancer cells including two multidrug-resistant (MDR) cell lines (IGROV-ET and LOVO-DOX) are shown in Table 2. According to these values some generalizations can be deduced: (i) the results show that although both series have antiproliferative activity in the micromolar range, compounds in series **4** are clearly more potent than compounds in series **3**. (ii) The role of intercalative binding in the cytotoxicity is presently unclear. Thus, the lack of intercalating capacity of **3c** seems to result in an appreciable decrease in cytotoxicity when compared with the DNA intercalator **3b**, but the capacity is similar to that found for **3a**, which also exhibits intercalative binding behavior. (iii) The cytotoxicity of DNA intercalators **4a**−**d** seems to correlate to some extent with their Topo1 inhibitory activity. Thus, the most potent compound (**4d**) completely inhibits the topoisomerase reaction, whereas **4a** and **4b** are more potent than **4c**, which does not inhibit this enzyme at all. Nevertheless, the mechanism of inhibition, as stated before, does not seem to point to this enzyme as the molecular target responsible for the cytotoxic effects. (iv) Although the substitution pattern with simple

Table 2. Antiproliferative Activity (GI_{50}, μM)[a] on Human Cancer Cells Lines of Compounds **3** and **4**

cell line		3a	3b	3c	3d	3e	4a	4b	4c	4d	Ad[b]
prostate	DU-145	NS	3.1	NS	4.8	1.8	1.0	0.5	5.8	0.5	0.02
	LN-caP	5.2	0.4	4.0	0.5	0.4	0.3	0.2	2.6	0.07	0.02
ovarian	IGROV	0.1	1.7	6.5	3.8	2.4	0.5	0.5	2.6	0.4	4.4
	IGROV-ET	NS	2.9	NS	8.6	4.5	2.0	1.8	NS	1.7	2.4
breast	SK-BR-3	9.7	0.5	4.6	1.2	0.5	0.5	0.5	3.9	0.5	1.1
melanoma	MEL-28	NS	2.5	8.4	2.6	1.2	1.0	0.5	5.3	0.4	3.1
NSCL	A-549	NS	4.2	NS	3.0	0.8	0.8	0.6	6.0	0.5	0.07
pancreas	PANC-1	NS	3.4	NS	2.1	1.0	1.3	0.7	4.9	0.4	3.5
colon	HT-29	NS	1.2	NS	1.1	0.6	0.4	0.4	5.4	0.2	3.1
	LOVO	NS	3.0	NS	2.9	1.0	0.6	0.4	6.3	0.3	0.01
	LOVO-DOX	NS	5.4	NS	8.0	7.0	3.5	3.6	NS	NS	2.4
cervix	HELA	NS	3.8	NS	3.1	1.3	0.7	0.5	4.6	0.3	0.01
	HELA-APL	NS	4.1	NS	5.0	1.4	0.1	NS	7.4	0.6	0.01
leukemia	K-562	7.6	1.5	NS	1.2	0.6	0.4	0.2	6.7	0.1	0.03

[a] GI_{50} = concentration that causes 50% growth inhibition. GI_{50} values higher than 10 μM are considered not significant (NS). All assays were performed in triplicate. [b] Ad = adriamycin.

electrodonating substituents seems to be critical for intercalation and Topo1 inhibition, it appears to be less relevant for activity in these series in contrast to what was observed with benzimidazolium and γ-carbolinium cations.[17−20] A methyl substituent does not significantly vary the activity in series **4** (**4a** vs **4b**, both DNA intercalators and Topo1 inhibitors) and moderately improves the cytotoxicity in series **3** (**3a** vs **3b**, both DNA intercalators). A comparison between **3b**/**3e** (both DNA intercalators) and **4b**/**4d** (both DNA intercalators and Topo1 inhibitors) to evaluate the role of the ethoxy substitution indicates that this group moderately increases the activity of **3e** and **4d** when compared to the unsubstituted derivatives **3b** and **4b**, respectively.

Conclusions

Isochromane was used as the starting material for the preparation of new azaquinolizinium-type cations. The synthesis was completed over seven steps and included as the key feature an intramolecular Westphal condensation. The resulting benzo[*f*]pyrido[2,1-*a*]phthalazinium and benzo[*f*]quino[2,1-*a*]phthalazinium salts displayed a broad spectrum of antiproliferative activity against human cancer cell lines and were evaluated for their ability to bind DNA through intercalation and their capacity to inhibit Topoisomerase I activity. Topo1 inhibition was associated only with the pentacyclic benzo[*f*]quino[2,1-*a*]phthalazinium cations, even though both cationic systems behaved as DNA intercalators. Theoretical calculations using density functional theory showed fused ring system **3** and **4** to be slightly nonplanar due to distortions induced by the phenyl substituent. Additional molecular modeling studies suggest a preferred orientation for the intercalating chomophores within CpG and TpG intercalation sites that resembles that found for the prototypical intercalator ethidium bromide.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer. General experimental conditions, such as absorbance measurements in the UV−visible region, were carried out as described previously.[18,20] 2-(2′-Bromoethyl)-benzaldehyde (**6**) was prepared according to the literature procedure.[24]

Theoretical Calculations. Ring systems **3** and **4** were model-built in Insight II[42] using standard bond lengths and angles. The initial geometries were first refined by means of the semiempirical quantum mechanics program MOPAC[43] and further optimized using hybrid density functional theory (B3LYP) and the 6-31G* basis set as implemented in the Gaussian program.[44] The AMBER all-atom force-field parameters for the chromophores were derived, by analogy or through interpolation, from those already present in the AMBER database,[45] and point charges that reproduced the molecular electrostatic potential were calculated with MOPAC (ESP keyword). Model complexes of the intercalators sandwiched at a $d(CG)_2$ and a $d(TG)_2$ dinucleotide step in all different orientations were built and simulated as described before.[20] Solvent polarization effects were implicitly included by use of a generalized Born model.[46]

General Procedure for Preparation of 7. To a suspension of the phosphonium salt (1 mmol) in dry THF (5 mL) at −78 °C in an atmosphere of dry argon was added *n*-BuLi (1 mmol, solution 1.6 M in hexanes), and the mixture was stirred for 1 h at the same temperature. A solution of **6** (X = Br, 1 mmol) in dry THF (5 mL) was added dropwise via cannula and the mixture was stirred for 2 h more. The reaction was quenched with $NaHCO_3$ solution, extracted with CH_2Cl_2, and dried ($MgSO_4$). Elimination of the solvent under reduced pressure and purification by flash chromatography [silica gel, hexane:ethyl acetate (9:1)] afforded a *Z*/*E* 1:1 mixture of alkenes **7**.

(*Z*/*E*)-2-(2′-Bromoethyl)stilbene (7a): A 1:1 mixture of alkenes; colorless oil (75%); IR (neat) ν_{max} 3061, 1724, 1599, 1494, 1447 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, 1H, J = 7.3 Hz), 7.53 (d, 2H, J = 7.6 Hz), 7.41−7.35 (m, 3H), 7.32−7.08 (m, 13H), 7.02 (d, 1H, J = 15.7 Hz), 6.70 (d, 2H, J = 2,0.9 Hz), 3.55 (t, 2H, J = 7.3 Hz), 3.54 (t, 2H, J = 7.3 Hz), 3.32 (t, 2H, J = 7.3 Hz), 3.20 (t, 2H, J = 7.3 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.2, 137.0, 136.8, 136.5, 136.4, 136.3, 131.5, 131.3, 130.0, 129.8, 129.6, 128.9, 128.7, 128.3, 128.1, 127.8, 127.7, 127.5, 127.4, 127.2, 127.0, 126.6, 126.2, 125.3, 37.2, 37.0, 31.9, 31.8 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 286, 288 (M^+, 36, 38), 178 (41), 90 (100); HRMS (EI) calcd for $C_{16}H_{15}$-Br (M^+) 286.0353, found 286.0376.

(*Z*/*E*)-2-(2′-Bromoethyl)-4″-ethoxystilbene (7b): A 1:1 mixture of alkenes; colorless oil (82%); IR (neat) ν_{max} 3060, 1606, 1511, 1476, cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (d, 1H, J = 7.8 Hz), 8.12 (d, 2H, J = 10.0 Hz), 7.97−7.80 (m, 10H), 7.68−7.55 (m, 5H), 7.34 (d, 2H, J = 8.8 Hz), 4.73 (q, 2H, J = 7.2 Hz), 4.63 (q, 2H, J = 7.0 Hz), 4.24−4.14 (m, 4H), 3.97 (t, 2H, J = 7.4 Hz), 3.83 (t, 2H, J = 7.4 Hz), 2.10 (t, 3H, J = 7.2 Hz), 2.03 (t, 3H, J = 7.0 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.1, 137.4, 136.8, 131.0, 130.2, 129.8, 129.6, 128.9, 127.3, 127.1, 126.2, 114.1, 63.3, 37.3, 32.0, 14.7 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 332, 330 (M^+, 26, 26), 107 (100), 93 (30), 77 (14); HRMS (EI) calcd for $C_{18}H_{19}BrO$ (M^+) 330.0638, found 330.0653.

(*Z*/*E*)-2-(2-Bromoethyl)-2-propenyl benzene (7c): A 1:1 mixture of alkenes; colorless oil (45%); IR (neat) ν_{max} 3064, 1603, 1511 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.04 (m, 8H), 6.51 (d, 2H, J = 12.0 Hz), 5.91−5.85 (m, 2H), 7.68−7.55 (m, 5H), 3.48 (t, 4H, J = 7.5 Hz), 3.15 (t, 4H, J = 7.5 Hz), 1.91 (d, 3H, J = 7.0 Hz), 1.71 (t, 3H, J = 7.0 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.0, 136.4, 135.5, 129.8, 129.7, 129.4, 128.4, 128.1, 127.8, 127.2, 127.0, 126.6, 126.3, 37.2, 37.0, 31.8, 31.7, 14.2 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 224, 226 (M^+, 30, 30), 93 (30), 77 (20); HRMS (EI) calcd for $C_{11}H_{13}$-Br (M^+) 224.0197, found 224.0221.

General Procedure for Preparation of 8 and 12. To a solution of the corresponding methylazine (2 mmol) in dry THF (5 mL) was added *n*-BuLi (1 mmol, solution 1.6 M in hexanes) dropwise at −20 °C under an atmosphere of dry argon. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. Then, a solution of **7** (1 mmol) in dry THF (5 mL) was added and stirring was continued at room temperature for 2 h more. The reaction mixture was quenched with $NaHCO_3$ solution, extracted with CH_2Cl_2, dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by flash chromatography [silica gel, hexane:ethyl acetate (8:2)], affording a *Z*/*E* 1:1 mixture of alkenes **8** and **12**, respectively.

(*Z*/*E*)-2-[3-(2-Styrilphenyl)propyl]pyridine (8a): A 1:1 mixture of alkenes; colorless oil (90%); IR (neat) ν_{max} 3064, 1738, 1594, 1462 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (dd, 2H, J = 10.6, 5.8 Hz), 7.65−6.99 (m, 26H), 6.71 (d, 1H, J = 11.1 Hz), 6.60 (d, 1H, J = 11.1 Hz), 2.94−2.82 (m, 6H), 2.74 (t, 2H, J = 7.69 Hz), 2.12−2.04 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.8, 149.2, 149.1, 140.9, 139.9, 136.8, 136.6, 136.1, 130.5, 130.1, 129.7, 129.3, 128.9, 128.5, 127.9, 127.5, 127.4, 127.3, 126.9, 126.5, 126.3, 125.8, 125.7, 122.7, 122.6, 120.8, 38.1, 37.9, 33.2, 32.8, 30.9, 30.4 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 299 (M^+, 21), 106 (100), 93 (58), 91 (65); HRMS (EI) calcd for $C_{22}H_{21}N$ (M^+) 299.1668, found 299.1666.

(*Z*/*E*)-2-Methyl-6-[3-(2-styrylphenyl)propyl]pyridine (8b): A 1:1 mixture of alkenes; colorless oil (62%); IR (neat) ν_{max} 3024, 1602, 1514, 1492. cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65−6.83 (m, 26H), 6.70 (d, 1H, J = 8.0 Hz), 6.57 (d, 1H, J = 10 Hz), 2.92−2.65 (m, 8H), 2.50 (s, 6H), 2.15−1.95 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.3, 164.0, 161.6, 149.0, 148.9, 147.1, 144.5, 140.2, 140.0, 137.6, 137.1, 136.9, 136.8,

136.6, 135.5, 130.5, 130.0, 129.7, 129.9, 128.9, 128.6, 128.0, 127.5, 127.4, 127.3, 126.9, 126.5, 126.2, 125.8, 125.6, 123.7, 123.5, 122.0, 121.9, 38.0, 37.8, 33.3, 32.9, 31.0, 30.5, 20.9 ppm; MS (EI, 70 eV) *m/z* (relative intensity) 313 (M^+, 27), 222 (100), 194 (24); HRMS (EI) calcd for $C_{23}H_{23}N$ (M^+) 313.1824, found 313.1830.

(*Z*/*E*)-4-Methyl-2-[3-(2-styrylphenyl)propyl]pyridine (8c). Following the general procedure, from 2,4-lutidine was obtained a mixture of regioisomers that were separated by flash chromatography (37%): a 1:1 mixture of alkenes; IR (neat) $\nu_{máx}$ 3024, 1602, 1514, 1492 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.38 (d, 1H, J = 6.2 Hz), 8.35 (d, 1H, J = 6.6 Hz), 7.60 (m 1H), 7.46 (d, 2H, J = 8.0 Hz), 7.38−6.90 (m, 21H), 6.68 (d, 1H, J = 12.1 Hz), 6.56 (d, 1H, J = 12.1 Hz), 2.85−2.76 (m, 6H), 2.69 (t, 2H, J = 7.32 Hz), 2.27 (s, 6H), 2.11−1.96 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.3, 164.0, 161.6, 149.0, 148.9, 147.1, 144.5, 140.2, 140.0, 137.6, 137.1, 136.9, 136.8, 136.6, 135.5, 130.5, 130.0, 129.7, 129.9, 128.9, 128.6, 128.0, 127.5, 127.4, 127.3, 126.9, 126.5, 126.2, 125.8, 125.6, 123.7, 123.5, 122.0, 121.9, 38.0, 37.8, 33.3, 32.9, 31.0, 30.5, 20.9 ppm; MS (EI, 70 eV) *m/z* (relative intensity) 313 (M^+,27), 222 (100), 120 (98), 91 (60); HRMS (EI) calcd for $C_{23}H_{23}N$ (M^+) 313.1824, found 313.1831.

(*Z*/*E*)-2-(3-{2-[2-(4-Ethoxyphenyl)vinyl]phenyl}propyl)-pyridine (8d): A 1:1 mixture of alkenes; colorless oil (97%); IR (neat) ν_{max} 3009, 1606, 1590, 1510, 1254 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.51 (dd, 2H, J = 5.5, 1.8 Hz), 8.53 (ddd, 1H, J = 7.6, 1.8 Hz), 7.25−7.15 (m, 8H), 7.09−6.99 (m, 9H), 6.69−6.64 (m, 6H), 6.54 (d, 1H, J = 12.1 Hz), 6.50 (d, 1H, J = 12.1 Hz), 3.97 (q, 2H, J = 6.9 Hz), 3.95 (q, 2, J = 6.9 Hz), 2.83 (m, 4H), 2.70 (t, 2H, J = 7.7 Hz), 2.08−1.97 (m, 4H), 1.36 (t, 3H, J = 6.96 Hz), 1.30 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.9, 161.9, 157.9, 157.6, 155.5, 149.1, 141.3, 141.2, 140.1, 137.0, 136.1, 130.9, 130.1, 129.9,129.7, 129.3, 128.8, 128.7, 127.5, 127.2, 127.1, 126.3, 125.8, 122.6, 120.8, 113.9, 63.2, 61.2, 38.1, 33.2, 30.3, 30.4, 22.0, 14.7 ppm; MS (EI, 70 eV) *m/z* (relative intensity) 343 (M^+, 5), 93 (100), 78 (10); HRMS (EI) calcd for $C_{24}H_{25}NO$ (M^+) 343.1929, found 343.1934.

(*Z*/*E*)-2-(3-{2-[2-(4-Ethoxyphenyl)vinyl]phenyl}propyl)-6-methylpyridine (8e): A 1:1 mixture of alkenes; colorless oil (98%); IR (neat) ν_{max} 3061, 1606, 1591, 1511, 1476, 1258 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 (t, 2H, J = 7.6 Hz), 7.26−7.16 (m, 4H), 7.09−7.01 (m 4H), 6.93 (d, 2H, J = 7.6 Hz), 6.88 (d, 2H, J = 7.6 Hz), 6.69−6.65 (m, 10H), 6.58 (d, 1H, J = 12.4 Hz), 6.51 (d, 1H, J = 12.4 Hz), 3.93 (q, 2H, J = 6.9 Hz), 3.90 (q, 2H, J = 6.9 Hz), 2.82 (m, 4H), 2.75 (t, 2H, J = 7.6 Hz), 2.72 (t, 2H, J = 7.6 Hz), 2.53 (s, 6H), 2.08−1.97 (m, 4H), 1.36 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.1, 157.8, 157.4, 145.5, 145.0, 144.9, 144.8, 147.1, 140.1, 140.0, 136.9, 136.2, 135.5, 135.4, 134.2, 131.9, 130.0, 129.9, 129.8, 129.2, 127.1, 127.0, 125.7, 120.2, 119.3, 113.9, 63.0, 61.1, 38.1, 33.2, 30.9, 30.6, 24.3, 14.6 ppm; MS (EI, 70 eV) *m/z* (relative intensity) 357 (M^+, 10), 107 (100), 92 (99); HRMS (EI) calcd for $C_{25}H_{27}NO$ (M^+) 357.2085, found 357.2097.

(*Z*/*E*)-2-[3-(2-Styrylphenyl)propyl]quinoline (12a): A 1:1 mixture of alkenes; colorless oil (75%); IR (neat) ν_{max} 3060, 1620, 1600, 1510, 694 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.08−8.01 (m, 2H), 7.77 (dd, 4H, J = 8.0, 2.5 Hz), 7.69 (tq, 2H, J = 8.8, 6.9, 1.8 Hz), 7.62−7.59 (m, 2H), 7.52−7.43 (m, 3H), 7.40 (dd, 2H, J = 8.4, 1.5), 7.34−7.20 (m, 9H), 7.17−6.96 (m, 8H), 6.70 (d, 1H, J = 12.4 Hz), 6.56 (d, 1H, J = 12.4 Hz), 3.11−3.01 (m, 4H), 2.89 (t, 2H, J = 7.6 Hz), 2.77 (t, 2H, J = 7.6 Hz), 2,20−2.11 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.3, 162.2, 147.8, 140.1, 139.9, 137.6, 136.8, 136.7, 136.2, 136.1, 135.9, 130.5, 130.2, 129.8, 129.3, 129.0, 128.9, 128.8, 128.5, 128.0, 127.6, 127.4, 127.3, 126.9, 126.7, 126.5, 126.3, 126.2, 125.9, 125.7, 121.3, 38.9, 38.7, 33.4, 33.0, 31.0, 30.5 ppm; MS (EI, 70 eV) *m/z* (relative intensity) 349 (M^+, 27), 143 (100), 91 (30), 77 (20); HRMS (EI) calcd for $C_{26}H_{23}N$ (M^+) 349.1824, found 349.1831.

(*Z*/*E*)-6-Methyl-2-[3-(2-styrylphenyl)propyl]quinoline (12b): A 1:1 mixture of alkenes; colorless oil (80%); IR (neat) ν_{max} 3058, 3022, 2943, 1599, 1495, 1481 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.95 (dd, 4H, J = 9.1, 6.9 Hz), 7.53−7.50 (m, 6H), 7.28−7.04 (m, 20H), 6.72 (d, 1H, J = 12.1 Hz), 6.57 (d, 1H, J = 12.1 Hz), 3.03 (m, 4H), 2.82 (t, 2H, J = 7.6 Hz), 2.78 (t, 2H, J = 7.69 Hz), 2.52 (s, 6H), 2.19−2.09 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.0, 159.5, 147.8, 146.6, 146.4, 145.5, 145.4, 143.9, 143.8, 142.7, 140.3, 140.1, 139.8, 139.6, 136.8, 136.6, 135.4, 135.3, 131.4, 130.5, 129.4, 129.3, 128.9, 128.4, 127.9, 127.3, 126.9, 126.6, 126.2, 125.8, 121.1, 38.8, 33.3, 30.5, 21.3, 14.1 ppm; MS (EI, 70 eV) *m/z* (relative intensity) 363 (M^+, 17), 157 (100), 91 (10), 78 (7); HRMS (EI) calcd for $C_{27}H_{25}N$ (M^+) 363.1980, found 363.1982.

(*Z*/*E*)-2-(3-{2-[2-(4-Ethoxyphenyl)vinyl]phenyl}propyl)-quinoline (12c): A 1:1 mixture of alkenes; colorless oil (88%); IR (neat) ν_{max} 3058, 1606, 1510, 1476, cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (dd, 4H, J = 8.4, 5.1 Hz), 7.75 (dd, 2H, J = 8.4, 1.5 Hz), 7.67 (ddd, 2H, J = 8.4, 6.6, 1.5 Hz), 7.47 (ddd, 2H, J = 8.1, 1.5 Hz), 7.25−7.16 (m, 10H), 7.10−6.98 (m 6H), 6.70−6.63 (m, 4H), 6.57 (d, 1H, J = 12.4 Hz), 6.47 (d, 1H, J = 12.4 Hz), 3.94 (q, 2H, J = 6.9 Hz), 3.91 (q, 2H, J = 6.9 Hz), 3.01 (m, 4H), 2.82 (t, 2H, J = 7.6 Hz), 2.74 (t, 2H, J = 7.6 Hz), 2.16−2.06 (m, 4H), 1.35 (t, 3H, J = 6.9 Hz), 1.31 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.4, 157.9, 156.9, 148.8, 148.7, 148.3, 147.8, 146.9, 146.5, 145.4, 144.3, 140.1, 137.1, 136.1, 131.9, 130.8, 130.1, 130.0, 129.7, 129.5, 129.3, 129.2, 128.8, 127.4, 127.2, 127.1, 126.7, 125.9, 125.6, 121.2, 113.9, 63.2, 59.9, 39.0, 33.4, 30.5, 30.1, 14.7 ppm; MS (EI, 70 eV) *m/z* (relative intensity) 393 (M^+, 10), 143 (100), 115 (20), 77 (10); HRMS (EI) calcd for $C_{28}H_{27}NO$ (M^+) 393.2085, found 393.2090.

(*Z*/*E*)-2-(3-{2-[2-(4-Ethoxyphenyl)vinyl]phenyl}propyl)-6-methylquinoline (12d): A 1:1 mixture of alkenes; colorless oil (74%); IR (neat) ν_{max} 3060, 1604, 1510, 1476, 1448 cm^{-1}; ^1H NMR (75 MHz, $CDCl_3$) δ 7.92 (d, 4H, J = 8.4 Hz), 7.52−7.48 (m, 4H), 7.25−7.16 (m, 8H), 7.08−6.99 (m, 8H), 6.6−6.64 (m, 4H), 6.56 (d, 1H, J = 12.1 Hz), 6.48 (d, 1H, J = 12.1 Hz), 3.96 (q, 2H, J = 6.9 Hz), 3.90 (q, 2H, J = 6.9 Hz), 2.99 (m, 4H), 2.82 (t, 2H, J = 7.6), 2.74 (t, 2H, J = 7.6 Hz), 2.51 (s, 6H), 2.17−2.07 (m, 4H), 1.35 (t, 3H, J = 6.9 Hz), 1.30 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.4, 157.9, 157.8, 148.9, 148.1, 147.3, 146.9, 146.3,141.2, 140.1, 137.0, 135.5, 135.3, 131.5, 130.1, 130.0, 129.8, 129.7, 129.6, 129.3, 128.4, 127.6, 127.2, 127.1, 126.7, 126.3, 125.9, 121.2, 114.6, 113.9, 63.2, 59.8, 38.8, 33.4, 30.5, 30.2, 21.4, 14.7 ppm; MS (EI, 70 eV) *m/z* (relative intensity) 407 (M^+, 10), 170 (50), 157 (100), 107 (25); HRMS (EI) calcd for $C_{29}H_{29}NO$ (M^+) 407.2241, found 407.2264.

General Procedure for Preparation of Diketones 9 and 13. To a solution of **8** or **12** (1 mmol) in DMSO (5 mL) was added HBr (1.2 mmol) and the mixture was heated for 12 h at 100 °C. Then, a $NaHCO_3$ solution was added until the pH was basic, the mixture was extracted with CH_2Cl_2 and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The crude products were purified by flash chromatography [silica gel, hexane:ethyl acetate (8:2)], yielding a pale yellow oil.

1-Phenyl-2-[2-(3-pyridin-2-yl-propyl)phenyl]ethane-1,2-dione (9a): 77%; IR (neat) ν_{max} 3065, 1719, 1678, 1596, 1448 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, 1H, J = 5.1 Hz), 7.96 (d, 2H, J = 7.7 Hz), 7.67−7.47 (m, 6H), 7.37 (d, 1H, J = 7.7 Hz), 7.26 (t, 1H, J = 7.7 Hz), 7.18 (d, 1H, J = 7.7 Hz), 7.09 (dd, 1H, J = 6.6, 5.1 Hz), 3.13 (t, 2H, J = 7.7 Hz), 2.91 (t, 2H, J = 7.7 Hz), 2.17−2.07 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.5, 194.4, 161.5, 148.7, 145.2, 136.3, 134.4, 133.5, 133.0, 131.7, 130.0, 129.9, 129.7, 128.8, 126.0, 122.7, 120.9, 37.8, 37.6, 31.1 ppm; MS (EI, 70 eV) *m/z* (relative intensity) 329 (M^+, 100), 242 (30), 224 (20), 123 (60); HRMS (EI) calcd for $C_{22}H_{19}NO_2$ (M^+) 329.1410, found 329.1434.

1-{2-[3-(6-Methylpyridin-2-yl)propyl]phenyl}2-phenyl-ethane-1,2-dione (9b): 71%; IR (neat) ν_{max} 3030, 1762, 1720, 1598, 1450 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.98−7.95 (m, 2H), 7.65 (tt, 1H, J = 6.6, 1.1 Hz), 7.60 (dd, 1H, J = 7.7, 1.5 Hz), 7.54−7.44 (m, 4H), 7.38 (dd, 1H, J = 7.7, 1.1 Hz), 7.25 (ddd, 1H, J = 7.7, 1.5 Hz), 6.96 (t, 2H, J = 8.1 Hz), 3.14 (t, 2H, J = 7.7 Hz), 2.87 (t, 2H, J = 7.7 Hz), 2.52 (s, 3H), 2.14−2.03 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.6, 194.6,

164.0, 161.1, 157.6, 145.5, 136.4, 134.6, 133.7, 133.2, 131.8, 131.6, 129.9, 128.9, 126.1, 120.4, 119.9, 38.3, 34.0, 31.4, 24.5 ppm; MS (EI) *m*/*z* (rel int) 343 (M^+, 2), 121 (100), 107 (99), 77 (30); HRMS (EI) calcd for $C_{23}H_{21}NO_2$ (M^+) 343.1575, found 343.1584.

1-{2-[3-(4-Methylpyridin-2-yl)propyl]phenyl}2-phenylethane-1,2-dione (9c): 43%; IR (neat) ν_{max} 3060, 1718, 1606, 1444 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, 1H, J = 8.1 Hz), 7.98−7.95 (m, 2H), 7.65 (tt, 1H, J = 6.6, 1.1 Hz), 7.69 (dd, 1H, J = 7.7, 1.5 Hz), 7.54−7.44 (m, 3H), 7.38 (dd, 1H, J = 7.7, 1.1 Hz), 7.25 (ddd, 1H, J = 7.7, 1.5 Hz), 7.00 (s, 1H), 6.91 (d, 1H, J = 8.1 Hz), 3.13 (t, 2H, J = 7.7 Hz), 2.86 (t, 2H, J = 7.7 Hz), 2.30 (s, 3H), 2.15−2.05 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.1, 193.0, 160.1, 154.6, 147.5, 135.4, 127.9, 127.8, 127.7, 127.1, 125.5, 124.8, 124.7, 124.5, 123.9, 122.4, 35.0, 33.7, 30.8, 20.9 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 343 (M^+, 2), 121 (100), 107 (99), 77 (39); HRMS (EI) calcd for $C_{23}H_{21}NO_2$ (M^+) 343.1575, found 343.1572.

1-(4-Ethoxyphenyl)-2-[2-(3-pyridin-2-yl-propyl)phenyl]-ethane-1,2-dione (9d): 61%; IR (neat) ν_{max} 3050, 1672, 1652, 1596, 1570 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.51 (d, 1H, J = 5.1 Hz), 7.93 (d, 2H, J = 8.8 Hz),7.61−7.55 (m, 2H), 7.47 (ddd, 1H, J = 7.7, 1.5 Hz), 7.35 (d, 1H, J = 7.7 Hz), 7.23 (ddd, 1H, J = 7.7, 2.2 Hz), 7.17 (d, 1H, J = 8.1 Hz), 7.09 (dd, 1H, J = 7.7, 5.1 Hz), 6.95 (d, 2H, J = 8.8 Hz), 4.11 (q, 2H, J = 6.9 Hz), 3.12 (t, 2H, J = 7.7 Hz), 2.90 (t, 2H, J = 7.7 Hz), 2.16−2.06 (m, 2H), 1.44 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.0, 193.3, 164.2, 164.0, 161.8, 149.1, 145.3, 136.2, 133.5, 133.2, 132.3, 131.9, 131.7, 126.0, 122.8, 120.9, 114.7, 63.9, 38.2, 33.9, 31.2, 14.5 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 373 (M^+, 2), 224 (100), 149 (99), 121 (40), 93 (32); HRMS (EI) calcd for $C_{24}H_{23}NO_3$ (M^+) 373.1671, found 373.1684.

1-(4-Ethoxyphenyl)-2-{2-[3-(6-methylpyridin-2-yl)propyl]-phenyl}ethane-1,2-dione (9e): 61%; IR (neat) ν_{max} 3030, 1666, 1595, 1454 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, 1H, J = 2.2 Hz), 7.81 (dd, 1H, J = 8.4, 2.2 Hz), 7.59 (dd, 1H, J = 8.1, 1.5 Hz), 7.49−7.42 (m, 3H), 7.34 (dd, 1H, J = 6.2, 1.5 Hz), 7.22 (ddd, 1H, J = 6.7, 1.1 Hz), 6.98−6.88 (m, 3H), 4.13 (q, 2H, J = 6.9 Hz), 3.12 (t, 2H, J = 7.7 Hz), 2.85 (t, 2H, J = 7.7 Hz), 2.57 (s, 3H), 2.12−2.02 (m, 2H), 1.45 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.9, 193.2, 164.0, 163.9, 161.8, 161.1, 157.5, 145.3, 136.4, 133.4, 133.0, 131.7, 131.4, 127.9, 125.9, 125.4, 120.3, 119.5, 111.1, 64.2, 38.2, 33.9, 31.4, 14.3, 14.5 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 387 (M^+, 10), 238 (90), 209 (100), 133 (50), 107 (22), 77 (12); HRMS (EI) calcd for $C_{25}H_{25}NO_3$ (M^+) 387.1827, found 387.1844.

1-Phenyl-2-[2-(3-quinolin-2-ylpropyl)phenyl]ethane-1,2-dione (13a): 75%; IR (neat) ν_{max} 3062, 1718, 1662 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.05 (t, 2H, J = 8.2 Hz), 7.98−7.95 (m, 2H), 7.77 (d, 1H, J = 8.2), 7.70−7.59 (m, 3H), 7.52−7.54 (m, 4H), 7.39 (d, 1H, J = 6.8 Hz), 7.34 (d, 1H, J = 8.4 Hz), 7.25 (t, 1H, J = 7.3 Hz), 3.20 (t, 2H, J = 7.7 Hz), 3.10 (t, 2H, J = 7.7 Hz), 2.27−2.19 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.7, 194.6, 162.3, 145.3, 136.1, 134.6, 133.7, 133.2, 133.1, 131.9, 131.6, 130.1, 129.8, 129.2, 128.9, 128.8, 127.4, 126.7, 126.1, 125.6, 121.4, 39.0, 34.1, 31.2 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 379 (M^+, 3), 274 (100), 256 (10), 143 (15), 128 (15), 105 (15), 77 (17); HRMS (EI) calcd for $C_{26}H_{21}NO_2$ (M^+) 379.1566, found 379.1584.

1-{2-[3-(6-Methylquinolin-2-yl)propyl]phenyl}2-phenylethane-1,2-dione (13b): 46%; IR (neat) ν_{max} 3060, 3024, 1718, 1672, 1446 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.98−7.92 (m, 4H), 7.64 (tt, 1H, J = 6.2, 1.1 Hz), 7.61 (dd, 1H, J = 7.7, 1.1 Hz); 7.52−7.47 (m, 4H), 7.38 (d, 1H, J = 6.6 Hz), 7.31 (s, 1H), 7.27 (d, 1H, J = 7.7 Hz), 7.25 (ddd, 1H, J = 7.7, 1.5 Hz), 3.19 (t, 2H, J = 7.7 Hz), 3.08 (2H, J = 7.7 Hz), 2.51 (s, 3H), 2.26−2.15 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.6, 194.6, 161.3, 146.4, 145.4, 135.6, 135.3, 134.7, 134.6, 133.2, 131.9, 131.6, 131.5, 130.0, 129.8, 128.9, 128.4, 126.7, 126.3, 126.1, 121.3, 38.9, 34.1, 31.2, 21.4 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 393 (M^+, 2), 374 (5), 288 (100), 105 (22), 77 (19); HRMS (EI) calcd for $C_{27}H_{23}NO_2$ (M^+) 393.1722, found 393.1728.

1-(4-Ethoxyphenyl)-2-[2-(3-quinolin-2-ylpropyl)phenyl]-ethane-1,2-dione (13c): 41%; IR (neat) ν_{max} 3060, 1734, 1658, 1506, 1428 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, 1H, J = 8.4 Hz), 7.87 (d, 1H, J = 2.2 Hz), 7.80 (dd, 1H, J = 8.8, 2.2 Hz), 7.75−7.71 (m, 1H), 7.67−7.59 (m, 2H), 7.48−7.41 (m, 3H), 7.36 (d, 1H, J = 7.7 Hz), 7.31 (d, 1H, J = 8.4 Hz), 7.25−7,19 (m, 2H), 6.84 (d, 1H, J = 8.8 Hz), 4.09 (q, 2H, J = 6.9 Hz), 3.18 (t, 2H, J = 7.7 Hz), 3.08 (t, 2H, J = 7.7 Hz), 2.21−2.12 (m, 2H), 1.42 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.0, 193.1, 162.2, 161.9, 147.8, 145.0, 136.0, 133.4, 133.0, 131.7, 131.6, 131.4, 129.1, 128.6, 128.4, 127.9, 127.3, 126.6, 125.5, 121.5, 111.0, 64.2, 38.9, 33.9, 31.1, 14.4 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 423 (M^+, 2), 274 (100), 156 (30); HRMS (EI) calcd for $C_{28}H_{25}NO_3$ (M^+) 423.1827, found 423.1844.

1-(4-Ethoxyphenyl)-2-{2-[3-(6-methylquinolin-2-yl)-propyl]phenyl}ethane-1,2-dione (13d): 41%; IR (neat) ν_{max} 3020, 1702, 1662, 1598 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, 2H, J = 8.4 Hz), 7.92 (d, 2H, J = 8.8 Hz), 7.61 (d, 1H, J = 7.7 Hz), 7.53−7.45 (m, 3H), 7.37 (d, 1H, J = 6.6 Hz), 7.30 (d, 1H, J = 8.4 Hz), 7.25 (ddd, 1H, J = 7.7, 1.5 Hz), 6.94 (d, 2H, J = 8.8 Hz), 4.10 (q, 2H, J = 6.9 Hz), 3.17 (t, 2H, J = 7.7 Hz), 3.06 (t, 2H, J = 7.7 Hz), 2.51 (s, 3H), 2.22−2.17 (m, 2H), 1.44 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.1, 193.3, 164.2, 161.4, 146.4, 145.3, 135.5, 135.3, 133.5, 133.2, 132.3, 131.9, 131.8, 131.5, 128.5, 126.4, 126.1, 126.0, 121.4, 114.7, 63.9, 39.0, 34.1, 31.3, 14.5 ppm; MS (EI, 70 eV) *m*/*z* (relative intensity) 437 (M^+, 2), 288 (100), 149 (30), 121 (20), 93 (72); HRMS (EI) calcd for $C_{29}H_{27}NO_3$ (M^+) 437.1977, found 437.1991.

General Procedure for Preparation of Salts 10 and 14. A solution of the dicarbonyl compound **9** or **13** (1 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of (*O*-mesitylensulfonyl)hydroxylamine (MSH, 1.5 mmol) in dichloromethane (5 mL) and the mixture was stirred at room temperature for 1 h. Diethyl ether was added (25 mL) and the precipitated solid was filtrated and recrystallized from absolute ethanol.

1-Amino-2-{3-[2-(2-oxo-2-phenylacetyl)phenyl]propyl}-pyridinium mesitylenesulfonate (10a): yellow solid (94%); mp 100−102 °C; IR (KBr) ν_{max} 3140, 2938, 2917, 1660, 1598, 1450, 1204 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 8.74 (d, 1H, J = 7.3 Hz), 8.23 (t, 1H, J = 8.4 Hz), 7.98 (d, 1H, J = 6.6 Hz), 7.92 (d, 2H, J = 8.4 Hz), 7.80 (t, 1H, J = 7.7 Hz), 7.72 (t, 1H, J = 7.7 Hz), 7.63−7.50 (m, 5H), 7.35 (t, 1H, J = 8.1 Hz), 6.85 (s, 2H), 3.29 (t, 2H, J = 7.7 Hz), 3.20 (t, 2H, J = 7.7 Hz), 2.60 (s, 6H), 2.22 (s, 3H), 2.20−2.14 (m, 2H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 195.5, 195.0, 164.3, 163.9, 163.2, 145.5, 142.9, 142.5, 140.1, 138.2, 136.2, 135.5, 134.7, 133.3, 131.6, 130.8, 130.3, 129.6, 127.9, 126.7, 34.5, 32.3, 29.1, 23.2, 20.8 ppm. Anal. Calcd for $C_{31}H_{32}N_2O_5S$: C, 68.36; H, 5.92; N, 5.14. Found: C, 68.07; H, 5.87; N, 5.05.

1-Amino-2-methyl-6-{3-[2-(2-oxo-2-phenylacetyl)phenyl]-propyl}pyridinium mesitylenesulfonate (10b): yellow solid (83%); mp 102−104 °C; IR (KBr) ν_{max} 3758, 3238, 3114, 2934, 1724, 1672, 1622, 1600 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 8.10 (ddd, 1H, J = 10.2, 7.7, 2.5 Hz), 8.00 (d, 1H, J = 7.7 Hz), 7.93−7.89 (m, 2H), 7.77−7.69 (m, 3H), 7.62−7.24 (m, 5H), 6.83 (s, 2H), 3.20 (t, 2H, J = 7.7 Hz), 2.89 (t, 2H, J = 7.7 Hz), 2.81 (s, 3H), 2.57 (s, 6H), 2.20 (s, 3H), 2.19−2.09 (m, 2H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 198.6, 196.3, 157.1, 145.5, 143.1, 140.1, 138.9, 136.2, 135.5, 134.7, 133.4, 132.5, 131.7, 130.7, 129.9, 129.4, 128.4, 127.9, 127.3, 127.2, 126.8, 34.5, 32.3, 29.4, 23.2, 20.8, 17.6 ppm. Anal. Calcd for $C_{32}H_{34}N_2O_5S$: C, 68.79; H, 6.13; N, 5.81. Found: C, 68.93; H, 6.32; N, 5.89.

1-Amino-4-methyl-2-{3-[2-(2-oxo-2-phenylacetyl)phenyl]-propyl}pyridinium mesitylenesulfonate (10c): yellow solid (76%); mp 118−120 °C; IR (KBr) ν_{max} 3082, 2974, 2934, 1664, 1634, 1598, 1450, 1208 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 8.56 (d, 1H, J = 6.9 Hz), 7.91 (t, 2H, J = 7.7 Hz), 7.80 (s, 1H), 7.70 (t, 1H, J = 8.0 Hz), 7.69−7.51 (m, 5H), 6.85 (s, 2H), 3.26 (t, 2H, J = 7.7 Hz), 3.20 (t, 2H, J = 7.7 Hz), 2.60 (s, 6H), 2.22 (s, 3H), 2.21−2.14 (m, 2H), 1.28 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 198.6, 196.3, 157.1, 145.6, 143.1, 140.1, 138.2,

136.2, 135.5, 134.7, 133.4, 132.5, 131.7, 130.7, 129.9, 129.4, 128.4, 127.9, 127.3, 127.2, 126.8, 34.5, 32.3, 29.4, 23.2, 21.4, 20.8 ppm. Anal. Calcd for $C_{32}H_{34}N_2O_5S$: C, 68.79; H, 6.13; N, 5.01. Found: C, 68.60; H, 6.20; N, 5.28.

1-Amino-2-(3-{2-[2-(4-ethoxyphenyl)-2-oxoacetyl]-phenyl}propyl)pyridinium mesitylenesulfonate (10d): yellow solid (68%); mp 108−110 °C; IR (KBr) ν_{max} 3452, 3106, 2980, 1664, 1600, 1568, 1264 cm^{-1}; ^{1}H NMR (300 MHz, CD_3-OD) δ 8.73 (d, 1H, J = 5.5 Hz), 8.19 (t, 1H, J = 8.0 Hz), 7.92 (d, 1H, J = 6.6 Hz), 7.87 (d, 2H, J = 8.8 Hz), 7.76 (t, 1H, J = 6.6 Hz), 7.61−7.54 (m, 2H), 7.48 (d, 1H, J = 7.0 Hz), 7.33 (t, 1H, J = 8.0 Hz), 7.04 (d, 2H, J = 8.8 Hz), 6.83 (s, 2H), 4.13 (q, 2H, J = 6.9 Hz), 3.25 (t, 2H, J = 7.7 Hz), 3.16 (t, 2H, J = 7.7 Hz), 2.59 (s, 6H), 2.20 (s, 3H), 2.19−2.10 (m, 2H), 1.40 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 198.3, 192.2, 166.1, 163.9, 142.9, 142.5, 140.8, 140.1, 138.1, 135.3, 134.5, 133.4, 133.2, 131.7, 129.6, 127.8, 126.9, 126.7, 123.2, 116.1, 115.7, 65.3, 34.4, 32.3, 29.1, 23.2, 20.8, 14.8 ppm. Anal. Calcd for $C_{33}H_{36}N_2O_3S$: C, 67.33; H, 6.16; N, 4.76. Found: C, 67.51; H, 6.27; N, 4.82.

1-Amino-2-(3-{2-[2-(4-ethoxyphenyl)-2-oxoacetyl]-phenyl}propyl)-6-methylpyridinium mesitylenesulfonate (10e): yellow solid (92%); mp 99−101 °C; IR (KBr) ν_{max} 3189, 2102, 1664, 1597, 1568, 1191 cm^{-1}; ^{1}H NMR (300 MHz, CD_3-OD) δ 8.12 (t, 1H, J = 8.1 Hz), 7.96−7.88 (m, 3H), 7.78−7.72 (m, 2H), 7.62−7.55 (m, 2H), 7.48 (d, 1H, J = 8.8 Hz), 7.33 (t, 1H, J = 7.7 Hz), 7.19 (d, 1H, J = 8.8 Hz), 6.84 (s, 2H), 4.22 (q, 2H, J = 6.9 Hz), 3.17−3.08 (m, 4H), 2.59 (s, 6H), 2.21 (s, 3H), 2.15−2.10 (m, 2H), 1.44 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 195.4, 193.5, 171.1, 168.1, 166.2, 163.3, 163.1, 162.7, 162.2, 161.2, 159.7, 156.0, 155.8, 155.4, 155.3, 154.8, 154.4, 154.3, 153.4, 141.1, 138.3, 63.2, 34.8, 30.5, 24.1, 23.3, 21.1, 20.8, 14.8 ppm. Anal. Calcd for $C_{34}H_{38}N_2O_6S$: C, 67.75; H, 6.35; N, 4.65. Found: C, 67.32; H, 6.34; N, 4.44.

1-Amino-2-{3-[2-(2-oxo-2-phenylacetyl)phenyl]propyl}-quinolinium mesitylenesulfonate (14a): yellow solid (92%); mp 97−99 °C; IR (KBr) ν_{max} 3428, 3064, 1672, 1602, 1248 cm^{-1}; ^{1}H NMR (75 MHz, CD_3OD) δ 9.33 (d, 1H, J = 8.8 Hz), 8.91 (d, 1H, J = 9.5 Hz), 8.59 (d, 1H, J = 9.5 Hz), 8.38 (dd, 1H, J = 8.1, 1.5 Hz), 8.24 (ddd, 1H, J = 8.8, 7.0, 1.5 Hz), 8.09 (t, 1H, J = 8.1 Hz), 7.87−7.80 (m, 2H), 7.67−7.54 (m, 3H), 7.49 (d, 1H, J = 7.0 Hz), 7.41 (ddd, 1H, J = 8.8, 7.0, 1.5 Hz), 7.09−7.02 (m 2H), 6.77 (s, 2H), 3.46 (t, 2H, J = 7.7 Hz), 3.24 (t, 2H, J = 7.7 Hz), 2.54 (s, 6H), 2.18 (s, 3H), 2.17−2.09 (m, 2H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 195.5, 194.6, 140.9, 139.9, 138.1, 137.1, 137.0, 135.5, 132.9, 132.3, 132.1, 131.6, 130.9, 130.8, 130.7, 130.5, 130.2, 129.8, 129.5, 129.4, 127.6, 120.4, 119.2, 27.6, 25.2, 24.2, 23.2, 20.8 ppm. Anal. Calcd for $C_{35}H_{34}N_2O_5S$: C, 70.69; H, 5.76; N, 4.71. Found: C, 70.72; H, 5.61; N, 4.51.

1-Amino-6-methyl-2-{3-[2-(2-oxo-2-phenylacetyl]phenyl]-propyl}quinolinium mesitylenesulfonate (14b): yellow solid (98%); mp 86−88 °C; IR (KBr) ν_{max} 3252, 1679, 1674, 1600, 1450, 1210 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 8.73 (dd, 1H, J = 8.8, 5.1 Hz), 8.51 (d, 1H, J = 9.1 Hz), 7.99−7.83 (m, 5H), 7.70 (t, 1H, J = 7.3 Hz), 7.61−7.24 (m, 6H), 6.77 (s, 2H), 3.22 (t, 2H, J = 7.7 Hz), 2.96 (t, 2H, J = 7.7 Hz), 2.60 (s, 3H), 2.54 (s, 6H), 2.23−2.20 (m, 2H), 2.18 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 197.5, 194.3, 144.8, 141.8, 140.8, 140.1, 138.3, 138:2, 138.1, 133.3, 132.8, 131.8, 131.6, 131.2, 130.8, 130.5, 130.4, 129.9, 129.5, 127.6, 127.4, 124.5, 120.2, 119.1, 110.3, 34.6, 33.9, 30.7, 23.2, 20.8, 18.3 ppm. Anal. Calcd for $C_{36}H_{36}N_2O_5S$: C, 71.03; H, 5.96; N, 4.60. Found: C, 70.95; H, 5.98; N, 4.59.

1-Amino-2-(3-{2-[2-(4-ethoxyphenyl)-2-oxo-2-acetyl]-phenyl}propyl)quinolinium mesitylenesulfonate (14c): yellow solid (75%); mp 106−108 °C; IR (KBr) ν_{max} 3156, 1663, 1599, 1446, 1189 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 8.82 (d, 1H, J = 8.4 Hz), 8.70 (d, 1H, J = 8.4 Hz), 8.23 (t, 2H, J = 7.7 Hz), 8.14 (t, 1H, J = 7.3 Hz), 8.05−7.86 (m, 5H), 7.60−7.56 (m, 1H), 7.51 (t, 1H, J = 5.5 Hz), 7.32 (t, 1H, J = 7.0 Hz), 7.23 (d, 1H, J = 8.0 Hz), 6.79 (s, 2H), 4.25 (q, 2H, J = 6.9 Hz), 3.55 (t, 2H, J = 7.7 Hz), 3.21 (t, 2H, J = 7.7 Hz), 2.55 (s, 6H), 2.19 (s, 3H), 2.25−2.11 (m, 2H), 1.46 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 196.7, 193.4, 143.9, 143.7, 143.6, 143.2, 140.0, 138.9, 138.3, 136.2, 134.4, 134.0, 133.6, 133.1, 133.0, 132.8, 131.5, 129.2, 129.1, 128.7, 126.1, 123.4, 117.6, 111.8, 63.9, 33.1, 32.9, 28.6, 21.5, 19.8, 19.0 ppm. Anal. Calcd for $C_{37}H_{38}N_2O_6S$: C, 69.57; H, 6.00; N, 4.39. Found: C, 69.32; H, 6.13; N, 4.57.

1-Amino-2-(3-{2-[2-(4-ethoxyphenyl)-2-oxo-2-acetyl]-phenyl}propyl)-6-methylquinolinium mesitylenesulfonate (14d): yellow solid (84%); mp 110−112 °C; IR (KBr) ν_{max} 3424, 2984, 1654, 1600, 1512, 1266 cm^{-1}; ^{1}H NMR (300 MHz, CD_3-OD) δ 8.77 (d, 1H, J = 8.7 Hz), 8.52 (d, 1H, J = 8.7 Hz), 8.01−7.98 (m, 2H), 7.92 (d, 1H, J = 8.4 Hz), 7.84 (d, 2H, J = 9.1 Hz), 7.59−7.48 (m, 3H), 7.31 (t, 1H, J = 7.3 Hz), 7.02 (d, 2H, J = 9.1 Hz), 6.79 (s, 2H), 4.13 (q, 2H, J = 6.9 Hz), 3.55 (t, 2H, J = 7.7 Hz), 3.21 (t, 2H, J = 7.7 Hz), 2.60 (s, 3H), 2.55 (s, 6H), 2.30−2.18 (m, 2H), 2.19 (s, 3H), 1.40 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, CD_3OD) 196.6, 195.0, 164.0, 163.9, 163.3, 145.3, 144.8, 141.8, 140.0, 138.3, 138.1, 135.2, 134.5, 133.4, 133.2, 131.6, 130.4, 129.7, 127.9, 124.6, 119.1, 116.0, 63.2, 34.7, 30.5, 24.0, 23.2, 21.1, 20.8, 14.8 ppm. Anal. Calcd for $C_{38}H_{40}N_2O_6S$: C, 69.92; H, 6.18; N, 4.29. Found: C, 69.80; H, 6.13; N, 4.50.

General Procedure for Preparation of Benzo[*f*]pyrido-[2,1-*a*]phthalazinium Mesitylenesulfonates 3 and Benzo-[*f*]quino[2,1-*a*]phthalazinium Mesitylenesulfonates 4. Sodium acetate (method A) or Et_3N (method B) (1.2 mmol) was added to a suspension of the pyridinium (**10**) or quinolinium (**14**) salts (1 mmol) in acetone (method A) or EtOH (method B) (20 mL) and the mixture was heated at reflux for 6 h. Then, the solvent was concentrated under vacuum, and the solid residue was washed with EtOAc and Et_2O and crystallized from EtOH/Et_2O.

5-Phenylbenzo[*f*]pyrido[2,1-*a*]phthalazin-7-ium mesitylenesulfonate (3a): method A; yellow solid (65%); mp 199−201 °C; IR (KBr) ν_{max} 3419, 2946, 1602, 1563, 1451, 1203 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 9.59 (d, 1H, J = 6.9 Hz), 9.51 (d, 1H, J = 9.5 Hz), 9.04 (d, 1H, J = 9.1 Hz), 8.78 (t, 2H, J = 9.1 Hz), 8.30 (t, 1H, J = 7.3 Hz), 8.26 (d, 1H, J = 9.1 Hz), 7.85−7.63 (m, 7H), 7.44 (t, 1H, J = 6.9 Hz), 6.79 (s, 2H), 2.60 (s, 6H), 2.23 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 142.9, 142.4, 140.8, 140.3, 140.1, 139.0, 138.9, 138.3, 138.2, 137.6, 132.0, 131.7, 131.4, 130.7, 130.6, 130.1, 129.4, 129.3, 129.0, 126.9, 124.6, 120.9, 23.3, 20.8 ppm. Anal. Calcd for $C_{31}H_{26}N_2O_3S$: C, 73.49; H, 5.17; N, 5.53. Found: C, 73.20; H, 4.98; N, 5.78.

5-Phenyl-8-methylbenzo[*f*]pyrido[2,1-*a*]phthalazin-7-ium mesitylenesulfonate (3b): method B; yellow solid (50%); mp 221−223 °C; IR (KBr) ν_{max} 2930; 1625, 1471, 1369, 1190, 1015 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 9.39 (d, 1H, J = 9.3 Hz), 9.00 (d, 1H, J = 8.7 Hz), 8.73 (d, 1H, J = 8.7 Hz), 8.63 (t, 1H, J = 8.1 Hz), 8.24−8.28 (m, 2H), 7.84−7.65 (m, 7H), 7.44 (t, 1H, J = 7.7 Hz), 6.79 (s, 2H), 3.17 (s, 3H), 2.54 (s, 6H), 2.18 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 143.1, 141.4, 140.8, 140.2, 140.0, 138.7, 138.2, 132.1, 131.6, 131.2, 130.9, 130.6, 130.3, 130.1, 129.5, 129.4, 129.1, 128.4, 128.0, 127.9, 127.7, 127.5, 127.4, 127.3, 23.2, 20.8, 18.3 ppm. Anal. Calcd for $C_{32}H_{28}N_2O_3S$: C, 73.82; H, 5.42; N, 5.38. Found: C, 74.11; H, 5.19; N, 5.40.

5-Phenyl-10-methylbenzo[*f*]pyrido[2,1-*a*]phthalazin-7-ium mesitylenesulfonate (3c): method B; yellow solid (28%); mp 222−224 °C; IR (KBr) ν_{max} 3028, 1632, 1405, 1194 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 9.33 (d, 1H, J = 6.6 Hz), 8.61 (s, 1H), 8.06 (d, 1H, J = 6.6 Hz), 7.80−7.32 (m, 9H), 7.00−6.91 (m, 2H), 6.83 (s, 2H), 2.82 (s, 3H), 2.59 (s, 6H), 2.21 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 143.7, 142.4, 140.6, 134.6, 134.4, 134.3, 134.0, 133.7, 133.6, 133.5, 133.1, 133.0, 132.7, 132.4, 132.3, 132.2, 132.1, 131.8, 131.7, 130.8, 129.8, 126.4, 27.2, 25.6, 23.2 ppm. Anal. Calcd for $C_{32}H_{28}N_2O_3S$: C, 73.82; H, 5.42; N, 5.38. Found: C, 73.98; H, 5.45; N, 5.23.

5-(4-Ethoxyphenyl)benzo[*f*]pyrido[2,1-*a*]phthalazin-7-ium mesitylenesulfonate (3d): method A; yellow solid (22%); mp 225−227 °C; IR (KBr) ν_{max} 2932, 1604, 1458, 1298 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 9.55 (d, 1H, J = 6.5 Hz), 9.43 (d, 1H, J = 8.0 Hz), 8.97 (d, 1H, J = 8.8 Hz), 8.73 (dd, 2H, J = 8.4, 6.9 Hz), 8.30−8.24 (m, 2H), 7.97 (d, 1H, J = 8.8 Hz),

7.82 (t, 1H, J = 8.0 Hz), 7.66−7.61 (m, 2H), 7.49 (t, 1H, J = 8.8 Hz), 7.15 (d, 1H, J = 8.8 Hz), 7.04 (t, 1H, J = 8.8 Hz), 6.79 (s, 2H), 4.19 (q, 2H, J = 6.9 Hz), 2.56 (s, 6H), 2.18 (s, 3H), 1.47 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 142.7, 142.0, 140.8, 138.7, 138.2, 132.6, 131.9, 131.6, 131.5, 130.7, 130.6, 130.0, 129.5, 129.4, 129.2, 129.1, 129.0, 127.5, 126.8, 126.7, 124.5, 116.4, 115.9, 30.4, 23.2, 20.8, 15.0 ppm. Anal. Calcd for $C_{33}H_{30}N_2O_4S$: C, 71.98; H, 5.49; N, 5.09. Found: C, 70.89; H, 5.30; N, 5.23.

5-(4-Ethoxyphenyl)-8-methylbenzo[*f*]pyrido[2,1-*a*]phthalazin-7-ium mesitylenesulfonate (3e): method B; yellow solid (28%); mp 180−182 °C; IR (KBr) ν_{max} 3438, 1604, 1467, 1388, 1189 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 9.31 (d, 1H, J = 9.3 Hz), 8.96 (d, 1H, J = 9.3 Hz), 8.71−8.59 (m, 1H), 8.42 (t, 1H, J = 7.6 Hz), 8.23 (d, 1H, J = 8.0 Hz), 8.14 (d, 1H, J = 7.6 Hz), 7.67 (d, 2H, J = 8.5 Hz), 7.45 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 7.6 Hz), 7.15 (d, 1H, J = 8.3 Hz), 7.02 (d, 2H, J = 8.5 Hz), 6.79 (s, 2H), 4.11 (q, 2H, J = 6.9 Hz), 3.17 (s, 3H), 2.55 (s, 6H), 2.18 (s, 3H), 1.42 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 143.3, 142.5, 140.6, 140.5, 134.7, 134.5, 134.2, 134.1, 133.8, 133.1, 133.0, 132.9, 132.6, 132.5, 132.4, 131.9, 131.5, 130.4, 130.3, 130.2, 130.1, 130.0, 125.6, 115.7, 30.2, 25.7, 23.2, 20.8, 11.6 ppm. Anal. Calcd for $C_{34}H_{32}N_2O_4S$: C, 72.32; H, 5.71; N, 4.96. Found: C, 72.09; H, 5.99; N, 4.90.

15-Phenylbenzo[*f*]quino[2,1-*a*]phthalazin-13-inium mesitylenesulfonate (4a): method A; yellow solid (75%); mp 280−282 °C; IR (KBr) ν_{max} 2930, 1600, 1507, 1398, 1353, 1205 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 9.40 (d, 1H, J = 8.8 Hz), 9.36 (d, 1H, J = 9.1 Hz), 9.15 (d, 2H, J = 9.1 Hz), 8.81 (d, 1H, J = 9.1 Hz), 8.47 (d, 1H, J = 8.0 Hz), 8.29 (t, 2H, J = 9.1 Hz), 8.12 (t, 1H, J = 8.0 Hz), 7.95 (d, 1H, J = 8.8 Hz), 7.83−7.90 (m, 2H), 7.69−7.78 (m, 3H), 7.50 (t, 2H, J = 7.3 Hz), 6.80 (s, 2H), 2.57 (s, 6H), 2.19 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 142.2, 140.9, 139.9, 139.5, 139.2, 139.0, 138.2, 138.0, 135.9, 132.2, 132.1, 131.6, 130.8, 130.3, 129.5, 129.4, 129.3, 129.2, 125.0, 121.5, 120.7, 118.8, 23.2, 20.7 ppm. Anal. Calcd for $C_{35}H_{28}N_2O_3S$: C, 75.52; H, 5.07; N, 5.03. Found: C, 75.77; H, 5.30; N, 4.88.

15-Phenyl-10-methylbenzo[*f*]quino[2,1-*a*]phthalazin-13-ium mesitylenesulfonate (4b): method B; yellow solid (50%); mp 220−222 °C; IR (KBr) ν_{max} 2927, 1601, 1448, 1188 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 9.52 (d, 1H, J = 8.8 Hz), 9.30 (d, 1H, J = 9.1 Hz), 9.18 (t, 2H, J = 9.1 Hz), 8.95 (d, 1H, J = 9.1 Hz), 8.38 (d, 1H, J = 8.0 Hz), 8.32 (s, 1H), 8.13 (d, 1H, J = 8.0 Hz), 7.90 (t, 1H, J = 8.8 Hz), 7.70−7.82 (m, 5H), 7.51−7.62 (m, 2H), 6.69 (s, 2H), 2.66 (s, 3H), 2.45 (s, 6H), 2.13 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 143.3, 141.7, 141.3, 140.6, 139.7, 138.9, 138.6, 137.9, 137.6, 137.4, 131.8, 131.3, 130.5, 130.1, 129.5, 129.4, 129.1, 129.0, 124.3, 121.2, 120.2, 118.5, 23.9, 21.1, 20.5 ppm. Anal. Calcd for $C_{36}H_{30}N_2O_3S$: C, 75.76; H, 5.30; N, 4.91. Found: C, 75.56; H, 5.23; N, 5.12.

15-(4-Ethoxyphenyl)benzo[*f*]quino[2,1-*a*]phthalazin-13-ium mesitylenesulfonate (4c): method B; yellow solid (28%); mp 198−200 °C; IR (KBr) ν_{max} 2933, 1604, 1190, 1088 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 9.36 (d, 1H, J = 8.8 Hz), 9.20 (t, 1H, J = 8.0 Hz), 9.00 (d, 1H, J = 8.8 Hz), 8.95 (t, 1H, J = 8.0 Hz), 8.32−8.22 (m, 4H), 8.02 (d, 2H, J = 8.8 Hz), 7.87 (t, 1H, J = 7.7 Hz), 7.70 (d, 2H, J = 8.4 Hz), 7.50 (t, 1H, J = 7.7 Hz), 7.17 (d, 2H, J = 8.0 Hz), 6.70 (s, 2H), 4.22 (q, 2H, J = 6.9 Hz), 2.60 (s, 6H), 2.20 (s, 3H), 1.60 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 160.9, 160.0, 150.0, 144.5, 141.6, 140.9, 139.8, 138.7, 138.0, 137.7, 137.6, 135.4, 132.5, 132.2, 131.7, 131.0, 129.7, 129.1, 124.5, 120.9, 120.4, 119.0, 118.7, 116.2, 112.2, 110.1, 65.2, 23.2, 21.4, 20.8 ppm. Anal. Calcd for $C_{37}H_{32}N_2O_4S$: C, 73.98; H, 5.37; N, 4.66. Found: C, 74.30; H, 5.23; N, 4.77.

15-(4-Ethoxyphenyl)-10-methylbenzo[*f*]quino[2,1-*a*]phthalazin-13-ium mesitylenesulfonate (4d): method B; yellow solid (50%); mp 220−222 °C; IR (KBr) ν_{max} 2947, 1603, 1507, 1452, 1372, 1249 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 9.16 (d, 2H, J = 8.8 Hz), 8.95 (t, 2H, J = 8.0 Hz), 8.67 (d, 1H, J = 8.8 Hz), 8.21 (d, 1H, J = 8.0 Hz), 8.15 (s, 1H), 8.02 (d, 2H, J = 8.8 Hz), 7.82 (t, 1H, J = 7.7 Hz), 7.70 (d, 2H, J = 8.4 Hz), 7.49 (t, 1H, J = 7.7 Hz), 7.17 (d, 2H, J = 8.0), 6.69 (s, 2H), 4.20 (q, 2H, J = 6.9), 2.58 (s, 3H), 2.50 (s, 6H), 2.13 (s, 3H), 1.49 (t, 3H, J = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 162.9, 160.6, 143.5, 141.6, 140.9, 139.8, 138.7, 138.2, 138.1, 137.7, 137.5, 132.1, 132.0, 131.5, 130.9, 130.6, 129.7, 129.6, 129.1, 124.5, 121.3, 120.4, 118.7, 116.6, 65.0, 23.2, 21.4, 20.8, 15.0 ppm. Anal. Calcd for $C_{38}H_{34}N_2O_4S$: C, 74.24; H, 5.57; N, 4.56. Found: C, 73, 91; H, 5.52; N, 4.37.

DNA Binding Experiments.

Electrophoretic Titration of Supercoiled Bacterial Plasmid DNA. Each reaction mixture, in a final volume of 6 μL, contained 200 ng of supercoiled bacterial plasmid DNA (pBluescript SK+, Stratagene, 2961 pb, equivalent final concentration 5.12 × 10^{-5} M base pairs), 5% glycerol, in 2× TAE (TAE: 40 mM Tris−acetic acid, 1 mM EDTA, pH 8) plus the compound under study at the final concentration indicated in each case. The complete reaction mixture was incubated for 1 h at 37 °C before electrophoresis, which was performed in 1× TAE, 1% agarose gel at 60 V (4.7 V/cm, 100 mA) for 1 h. Gels are stained in 5 μg/mL ethidium bromide in water and visualized using a UV transilluminator. The plasmid DNA was prepared using the Wizard Plus Midipreps DNA Purification System (Promega, Cat.# A7640; http://www.promega.com), following its recommended instructions, from 200 mL exponential phase cultures of *E. coli* Dh5a carrying the artificial cloning vector plasmid pBluescript SK+ (Stratagene). Bacterial growth was carried out at 37 °C in Luria-Bertani (LB) broth [10 g of Bacto-Tryptone (Difco 0123-01-1), 5 g of Bacto-yeast extract (Difco 0127-05-3) 10 g of NaCl, doubly distilled H_2O to 1 L] supplemented with 100 mg/mL ampicillin.[47−49]

Electrophoretic Assay of Supercoiled and Relaxed Bacterial Plasmid DNA. The complete assay involves four sample mixtures (1−4) and two agarose gels. Each mixture, in a final volume of 24 μL, contains 800 ng of bacterial plasmid DNA (pBluescrips SK+, Stratagene, 2961 pb, equivalent final concentration 5.12 × 10^{-5} M base pairs), 5% glycerol, and 2× TAE. Mixtures 1 and 3 were prepared from untreated supercoiled plasmid, whereas mixtures 2 and 4 were prepared from a DNA topoisomerase I-relaxed, covalently closed plasmid. Finally, mixtures 3 and 4 were supplemented with the compound under study at a final concentration of 0.1 mM and incubated for 1 h at 37 °C before electrophoresis. Samples of 6 μL from each mixture were subjected to electrophoresis in two independent 1× TAE, 1% agarose gels at 60 V (4.7 V/cm, 100 mA) for 1 h. One of the two gels contained the compound under study in the mass of the agarose gel at a final concentration of 0.01 mM. Both gels were postelectrophoretically stained with 5 μg/mL ethidium bromide in water and visualized using an UV transilluminator.

UV−Visible Spectrophotometric Determination. Calf thymus DNA for spectrophotometric binding analysis was purchased from Sigma Chemical Co. DNA concentrations were determined by absorbance measurements using an extinction coefficient of 13 200 M^{-1} cm^{-1} at 260 nm and are expressed in terms of base pairs equivalents per liter. The aqueous solution used was TRIS buffer (50 mM Tris, 15 mM NaCl, pH 7.6).

All compounds examined obeyed Beer's law over the range of concentrations used (0−20 μM), and the molar extinction coefficients were determined at their appropriate λ_{max} values by Beer's law plots. Molar extinction coefficients of compounds bound to DNA were determined at the same wavelength as the molar extinction coefficient measurements of the free compound, but a larger molar excess of DNA was present [DNA nucleotides]/[compound] > 20−100). Spectrophotometric titrations were performed by serial addition of 100 μL aliquots of corresponding compound **3** or **4** (6−20 μM) into a 10 cm path length quartz cell containing a DNA stock solution (0.1−0.5 mM DNA nucleotides in TRIS buffer) in the same buffer and scanning the UV−visible spectrum after each addition. Titrations were stopped when no shift to the lower energy range of the maximum absorbance wavelength in the spectrum was detected between additions. These absorbance values were converted to v (mol of bound compound/mol of DNA base pairs) and c (free ligand concentrations) using the free and bound

molar extinction coefficients for the analyzed compound. Experimental data that were in the fraction-bound range of 0.2−0.8 were plotted using the method of Scatchard (results outside of this range are subject to large systematic errors as a result of experimental errors in molar extinction coefficient determinations[50]). The salts **3** and **4** were analyzed by the extended neighbor-exclusion model of McGhee and von Hippel[32] using a nonlinear least-squares fitting procedure to derive the binding parameters (K, n) with a computer program[51] from the following equation:

$$v/c = K(1 - nv)\,[(1 - nv)/(1 - (n - 1)v)]^{n-1}$$

DNA Topoisomerase I Inhibition Assay. Each reaction mixture, in a final volume of 20 μL, contained 340 ng of supercoiled bacterial plasmid DNA (pBluescrips SK+, Stratagene), 0.01% bovine serum albumin (BSA), in topoisomerase I reaction buffer (35 mM Tris-HCl pH 8.0, 72 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 5 mM spermidine), plus 1 unit of bovine DNA topoisomerase I (Amersham Pharmacia Biotech), and the compound under study at the final concentration indicated in each case. The complete reaction mixture was incubated for 1 h at 37 °C. The reaction was stopped by the addition of 20 μL of 1% sodium dodecyl sulfate. Half of the resulting volume (20 μL) of each mixture was subjected to electrophoresis in 1× TAE 1% agarose gel at 60 V (4.7 V/cm, 100 mA) for 1 h, and the gel was then stained postelectrophoretically with 5 μg/mL ethidium bromide in water. The remaining 20 μL of each mixture was subjected to electrophoresis under the same conditions but in an agarose gel supplemented with ethidium bromide at a final concentration of 0.1 mg/mL, without postelectrophoretic staining. The bands were visualized using an UV transilluminator. The first two lanes of each gel, labeled as C1 and C2, contained supercoiled and completely relaxed plasmids, respectively, as controls. The following lanes contained plasmid DNA treated with topoisomerase I in the presence of different concentrations of the compound under study (routinely 10, 5, and 1 μM).

In Vitro Cytotoxicity. A colorimetric type of assay, using the sulforhodamine B (SRB) reaction, has been adapted for a quantitative measurement of cell growth and viability, following the technique previously described.[52−55] Cells are seeded in 96-well microtiter plates, at 5×10^3 cells per well in aliquots of 195μL of RPMI medium, and they are allowed to attach to the plate surface by growing in drug-free medium for 18 h. Afterward, samples are added in aliquots of 5 μL (dissolved in DMSO/H_2O 3:7). After 72 h exposure, the antitumor effect is measured by the SRB methodology: cells are fixed by adding 50 μL of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubating for 60 min at 4 °C. Plates are washed with deionized water and dried; 100 μL of SRB solution (0.4wt %/vol in 1% acetic acid) is added to each microtiter well and incubated for 10 min at room temperature. Unbound SRB is removed by washing with 1% acetic acid. Plates are air-dried and bound stain is solubilized with Tris buffer. Optical densities are read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analysis is generated automatically by LIMS implementation, and some parameters for cellular responses are calculated. Using control OD values (C), test OD values (T), and time zero OD values (T_0), GI_{50} is calculated from $100 \times [(T - T_0)/C - T_0)] = GI_{50}$. GI_{50} data allowed us to predict that not only could a compound be cystostatic but it could also have a potential in terms of tumor reduction.

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References

(1) (a) Neidle, S. Recent Developments in Triple-Helix Regulation of Gene Expression. *Anti-Cancer Drug Des.* **1997**, *12*, 433−442.
(2) *Cancer Chemotherapeutic Agents*; Foye, W. O., Ed.; ACS: Washington, DC, 1995.
(3) Neidle, S.; Thurston, D. E. In *New Targets for Cancer Chemotherapy*; Kerr, D. J., Workman, P., Eds.; CRC Press: Boca Raton, FL, 1994.
(4) *Nucleic Acid Targeted Drug Design*; Propst, C. L., Perun, T. L., Eds.; Marcel Dekker: New York, 1992.
(5) Lerman, L. S. Structural Considerations in the Interaction of Deoxyribonucleic Acid and Acridines. *J. Mol. Biol.* **1961**, *3*, 18−30.
(6) Lerman, L. S. The Structure of the Deoxyribonucleic Acid (DNA)−Acridines Complex. *Proc. Natl. Acad. Sci. U.S.A* **1963**, *49*, 94−102.
(7) Miller, K. J.; Newlin, D. D. Interactions of Molecules with Nucleic Acids. VI. Computer Design of Chromophoric Intercalating Agents. *Biopolymers* **1982**, *21*, 633−652.
(8) Wilson, W. D.; Jones, R. in *Intercalation Chemistry*; Whittingham, M. S., Jacobson, A. J., Eds.; Academic Press: New York, 1981; Chapter 14.
(9) Denny, W. A.; Baguley, B. C.; Cain, B. F.; Waring, M. J. In *Mechanism of Action of Anticancer Drugs*; Neidle, S., Waring, M. J., Eds.; MacMillan: London, 1983.
(10) Gago, F. Stacking Interactions and Intercalative DNA Binding. *Methods: Companion Methods Enzymol.* **1998**, *14*, 277−292.
(11) Boger, D. L.; Fink, B. E.; Brunette, S. R.; Tse, W. C.; Hedrick, M. P. A Simple, High-Resolution Methods for Establishing DNA Binding Affinity and Sequence Selectivity. *J. Am. Chem. Soc.* **2001**, *123*, 5878−5891.
(12) Dervan, P. B. Molecular Recognition of DNA by Small Molecules. *Bioorg. Med. Chem.* **2001**, *9*, 2215−2235.
(13) Nakatani, K.; Matsuno, T.; Adachi, K.; Hagihara, S.; Saito, I. Selective Intercalation of Charge Neutral Intercalators into GG and CG Steps: Implication of HOMO−LUMO Interaction for Sequence-Selective Drug Intercalation into DNA. *J. Am. Chem. Soc.* **2001**, *123*, 5695−5702.
(14) Antonini, I.; Polucci, P.; Magnano, A.; Martelli, S. Synthesis, Antitumor Cytotoxicity and DNA-Binding of Novel *N*-5,2-Di(*ω*-aminoalkyl)-2,6-dihydropyrazolo[3,4,5-*kl*]-acridine-5-carboxamides. *J. Med. Chem.* **2001**, *44*, 3329−3333.
(15) Crow, S. D. G.; Bailly, C.; Garbay-Jaureguiberry, C.; Roques, B.; Shaw, B. R.; Waring, M. J. DNA Sequence Recognition by the Antitumor Drug Ditercalinium. *Biochemistry* **2002**, *41*, 8672−8682.
(16) Antonini, I.; Polucci, P.; Magnano, A.; Gatto, B.; Palumbo, M.; Menta, E.; Pescalli, N.; Martelli, S. 2,6-Di(*ω*-aminoalkyl)-2,5,6,7-tetrahydropyrazolo[3,4,5-*mn*]pyrimido-[5,6,1-*de*]acridine-5,7-diones: Novel, Potent, Cytotoxic, and DNA-Binding Agents. *J. Med. Chem.* **2002**, *45*, 696−702.
(17) Pastor, J.; Siró, J.; García Navío, J. L.; Vaquero, J. J.; Rodrigo, M. M.; Ballesteros, M.; Alvarez-Builla, J. Synthesis of New Azino Fused Benzimidazolium Salts. A New Family of DNA Intercalating Agents. 1. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 3043−3048.
(18) Pastor, J.; Siró, J.; García Navío, J. L.; Vaquero, J.; Alvarez-Builla, J.; Gago, F.; Pascual-Teresa, B.; Pastor, M.; Rodrigo, M. Azino-Fused Benzimidazolium Salts as DNA Intercalating Agents 2. *J. Org. Chem.* **1997**, *62*, 5476−5483.
(19) Molina, A.; Vaquero, J. J.; García Navío, J. L.; Alvarez-Builla, J.; Rodrigo, M. M.; Castaño, O.; Andrés, J. L. J. Azonia Derivatives of the *γ*-Carboline System, a New Class of DNA Intercalators. *Bioorg. Med. Chem. Lett.* **1996**, *13*, 1453−1456.
(20) Molina, A.; Vaquero, J. J.; García Navío, J. L.; Alvarez-Builla, J.; Pascual-Teresa, B.; Gago, F.; Rodrigo, M. M. Novel DNA Intercalators Based on the Pyrazino[1′,6′:1,2]pyrido[4,3-*b*]indol-5-inium System. *J. Org. Chem.* **1999**, *64*, 3907−3915.
(21) Siro, J. G. The Westphal Reaction: Synthesis of Novel DNA Intercalating Agents. Thesis, University of Alcalá, 1998.
(22) Vaquero, J. J.; Alvarez-Builla, J. Cycloimmonium Salts and their Ylides and Related Compounds: More than just 1,3-Dipoles. *Advances in Nitrogen Heterocycles*; Moody, C. J., Ed.; Jai Press: Stamford, CT, 2000; Vol. 4, pp 159−250.
(23) Martinez-Barrasa, V.; Burgos, C.; Izquierdo, M. L.; Alvarez-Builla, J.; Vaquero, J. J. The First Example of an Intramolecular Westphal Reaction. Synthesis of a New Aza-Quinolizinium Type System. *Tetrahedron Lett.* **1999**, *40*, 4115−4118.
(24) Rieche, A.; Schmitz, E. Isochroman, I. Ring Opening and Ring Clousure with Isochroman Derivatives. Two New Rearrangements. *Chem. Ber.* **1956**, *89*, 1254−1262.
(25) Yusubov, M. S.; Filimonov, V. D. Iodine in Dimethyl Sulfoxide as a New General Reagent for the Preparative Oxidation of 1,2-Diarylethenes and 1,2-Diarylethynes to Aromatic 1,2-Diketones. *Synthesis* **1991**, 131−132.
(26) Chi, K. W.; Yusubov, M. S.; Filimonov, V. D. Palladium Catalyst in DMSO for the Oxidation of Tolans to Benzils. *Synth. Commun.* **1994**, *24*, 2119−2122.

(27) Yusubov, M. S.; Filimonov, V. D.; Vasilyeva, V. P.; Chi, K. W. Chemoselective Oxidation of Carbon−Carbon Double or Triple Bonds to 1,2-Diketones with DMSO−Based Reagents. *Synthesis* **1995**, 1234−1236.
(28) Katritzky, A. R.; Wang, Z.; Lang, H.; Feng, D. Novel and Facile Syntheses of Alkenyl, Alkynyl and Aryl 1,2-Diketones. *J. Org. Chem*. **1997**, *62*, 4125−4130.
(29) Pelaprat, D.; Delbarre, A.; Le Guen, Le Pecq, J. B.; Roques, B. P. DNA Intercalating Compounds as Potential Antitumor Agents. 2. Preparation and Properties of 7H-pyridocarbazole Dimers. *J. Med. Chem*. **1980**, *23*, 1336−1346.
(30) Dougherty, G.; Pigram, W. J. Spectroscopic Analysis of Drug-nucleic Acid Interactions. *CRC Crit. Rev. Biochem*. **1982**, *12*, 103−132.
(31) Berman, H. M.; Young, P. R. The Interaction of Intercalating Drugs with Nucleic Acids. *Annu. Rev. Biophys. Bioeng*. **1981**, *10*, 87−114.
(32) McGhee, J. D.; von Hippel, P. H. Theorical Aspects of DNA-Protein Interactions. Cooperative and Noncooperative Binding of Large Ligands to a One-Dimensional Homogeneous Lattice. *J. Mol. Biol*. **1974**, *86*, 469−489.
(33) Fairley, T. A.; Tidwell, R. R.; Donkor, I.; Naiman, N. A.; Ohemeng, K. A.; Lombardy, R. J.; Bentley, J. A.; Cory, M. Structure, Dna Minor Gloove Binding, and Base Pair Specificity of Alkyl and Aryl-Linked bis(amidinobenzimidazoles) and bis-(amidinoindoles). *J. Med. Chem*. **1993**, *36*, 1746−1753.
(34) Cory, M.; Tidwell, R. R.; Fairley, T. A. Structure and DNA Binding Activity of Analogues of 1,5-Bis(4-amidinophenoxi)-pentane (Pentamidine). *J. Med. Chem*. **1992**, *35*, 431−438.
(35) Graves, D. E.; Watkins, C. L.; Yielding, L. W. Ethidium Bromide and its Photoreactive analogues: Spectroscopic Analysis of Deoxyribonucleic Acid Binding Properties. *Biochemistry* **1981**, *20*, 1887−1892.
(36) Kobayashi, N.; Fukuda, T.; Ueno, K.; Ogino, H. Extremely Non-Planar Phthalocyanines with Saddle or Helical Conformation: Synthesis and Structural Characterizations. *J. Am. Chem. Soc*. **2001**, *123*, 10740−10741.
(37) Wang, J. C. DNA Topoisomerases. *Annu. Rev. Biochem*. **1996**, *6*, 635−692.
(38) Stewart, L.; Redinbo, M. R.; Qiu, X.; Hol, W. G. J.; Champoux, J. J. A Model for the Mechanism of Human Topoisomerase I. *Science* **1998**, *279*, 1534−1541.
(39) Wang, J. C. Cellular Roles of DNA Topoisomerases: A Molecular Perspective. *Nat. Rev. Mol. Cell Biol*. **2002**, *3*, 430−440.
(40) Hsiang, Y. H.; Hertzberg, R.; Hecht, S.; Liu, L. F. Camptothecin Induces Protein-linked DNA Breaks via Mammalian DNA Topoisomerase I. *J. Biol. Chem*. **1985**, *260*, 14873−8.
(41) Bailly, C.; Dassonneville, L.; Colson, P.; Houssier, C.; Fukasawa, K.; Nishimura, S.; Yoshinari, T. Intercalation into DNA Is Not Required for Inhibition of Topoisomerase I by Indolocarbazole Antitumor Agents 1. *Cancer Res*. **1999**, *59*, 2853−2860.
(42) *Insight II, release 98.0* (1998), Molecular Simulations, 9685 Scranton Road, San Diego, CA 92121-3752.
(43) Stewart, J. J. P. *MOPAC 93* Fujitsu Ltd., Tokyo, Japan, 1993.
(44) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.11.2; Gaussian, Inc.: Pittsburgh, PA, 2001.
(45) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. A Second Generation Force Field for the Simulation of Proteins, Nucleic Acids, and Organic Molecules. *J. Am. Chem. Soc*. **1995**, *117*, 5179−5197.
(46) Tsui, V.; Case, D. A. Molecular Dynamics Simulations of Nucleic Acids with a Generalized Born Solvation Model. *J. Am. Chem. Soc*. **2000**, *122*, 2489−2498.
(47) *Current Protocols in Molecular Biology*; Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K., Eds.; John Wiley & Sons: New York, 1999.
(48) *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, 4th ed.; Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K., Eds.; John Wiley & Sons: New York, 1999.
(49) *Molecular Cloning, a Laboratory Manual*, 2nd ed.; Sambrook, J., Fritsch, E. F., Maniatis, T., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 1989; Vol. 1, pp 2, 3.
(50) Deranleau, D. A. Theory of Measurement of Weak Molecular Complexes. I. General Considerations. *J. Am. Chem. Soc*. **1969**, *91*, 4044−4049.
(51) Hintze, J. L. *BMPD*, Statistical Software Inc., Los Angeles, CA, 1991.
(52) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer drug screening. *J. Natl. Cancer Inst*. **1990**, *82* (13), 1107−1112.
(53) Faircloth, G. T.; Stewart, D.; Clement, J. J. A simple Screening Procedure for the Quantitative Measurement of Cytotoxicity Assay. *J. Tissue Culture Methods* **1988**, *11* (4), 201−205.
(54) Monks, A.; Scudiero, D.; Skehan, Ph.; Shoemaker, R.; Paull, K.; Vistica, D., Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolf, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of a High-flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. *Articles* **1991**, *83* (11), 757−766.
(55) Mosmann, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods* **1983**, *65*, 55−63.

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