Synthesis, Biological Activity, and Quantitative Structure−Activity Relationship Study of Azanaphthalimide and Arylnaphthalimide Derivatives

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A series of quinoline derivatives as aza analogues of the naphthalene chromophore and a series of "nonfused" tricyclic aromatic systems, in particular 5-arylquinolines and 5- or 6-aryl and heteroaryl naphthalene systems, were synthesized and evaluated for growth-inhibitory properties in several human cell lines. The analysis of quantitative structure−antitumor activity relationships for the growth-inhibitory properties is also reported. Findings suggest that these compounds may not express their cytotoxicity via interaction on DNA.

Introduction

DNA-intercalating antitumor drugs constitute an important class of drugs in anticancer therapy.[1] Naphthalimides are significant examples[2] that include compounds in clinical trials such as the mononaphthalimide amonafide **1**[3] and the bis(naphthalimide) LU 79553 (elinafide) **2**.[4] Nowadays, it is accepted that the antitumor activity of amonafide is closely related to its ability to stabilize the DNA−intercalator−topoisomerase II ternary complex.[5] Elinafide is not a poison of topoisomerase II (topo II). It is a strong DNA binder, but it does not stabilize the topo II−DNA complex (or binds very weakly).[6] As part of a program to broaden the scope of **1** and **2**, we have recently reported[7,8] the synthesis and biological evaluation of a new series of mono- and bis-intercalating agents where heterocyclic systems have been "fused" to the naphthalimide chromophore.

Previous studies have reported the consequences of appending "nonfused" aromatic systems. The results obtained by the Cheng group[9] with the "2-phenylnaphthalene-type" structural pattern hypothesis and by Denny and co-workers with tricyclic carboxamides[10] without a completely fused chromophore encouraged us to synthesize the structures proposed herein. In the present paper we describe the synthesis of new types of structures related to **1** and **2**. The first series has a quinoline nucleus as aza analogues of the naphthalene chromophore, **11**−**17**. They were designed to determine the effect of replacing carbon with nitrogen on antitumor activity. On the other hand, several series of "nonfused" tricyclic aromatic systems, 5- or 6-aryl and heteroarylnaphthalene systems **18**−**22**, were also prepared. The cytotoxic activity and the quantitative structure−activity relationship (QSAR) analysis for the growth-

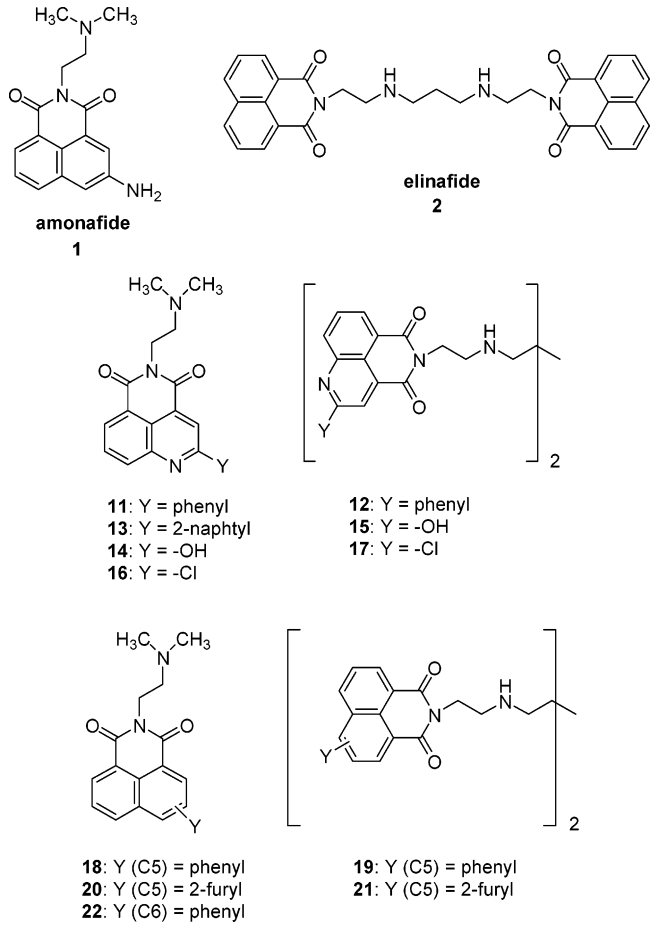

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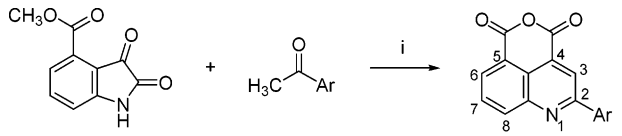
Table 1. Growth-Inhibitory Properties for Azanaphathlimide and Arylnaphathalimide Derivatives **11**−**22**

	IC_{50} [a]		
compd	HT-29	HeLa	PC-3
11	11.70	3.37	NT[b]
12	3.44	3.09	NT[b]
13	15.17	10.00	NT[b]
14	>100	>100	NT[b]
15	>100	>100	NT[b]
16	5.70	6.80	14.05
17	0.16	0.27	5.42
18	4.81	2.64	8.70
19	**0.021**	0.23	1.10
20	1.20	0.63	2.64
21	3.60	1.53	6.42
22	5.65	4.17	16.50
amonafide	4.67	2.73	6.38
elinafide	0.017	0.07	0.32

[a] IC_{50}: concentration of drug (μM) to reduce the cell number to 50% of control cultures. [b] NT: not tested.

Scheme 1[a]



[a] Reagents: (i) 85% KOH, EtOH, 80 °C, 4−5 days.

inhibitory properties in a panel of tumor cell lines are included in this study. Two compounds were selected for the assay of single-cell gel electrophoresis (comet assay) to further evaluate DNA damage. One of them, **16**, was selected because in QSAR studies it was found to be an outlier. The other was **19** because it emerges as the most potent among the new derivatives.

Results and Discussion

Chemistry. The synthesis of the compounds listed in Table 1 required the preparation of the corresponding anhydrides, which were obtained by the methods outlined in Schemes 1−3. The synthesis of some quinoline-based compounds involved an adaptation of the Pfitzinger synthesis,[11] in which the methyl isatin-4-carboxylate **1** reacted with a ketone to give the anhydrides **2** and **3** (Scheme 1). Many variations of the base catalysis have been employed[12,13] in the Pfitzinger reaction, and we obtained **2** and **3** with 85% potassium hydroxide at 80 °C for 4−5 days,[14] although the yield for each was less than 25%. Both compounds showed a spontaneous loss of water to give the final anhydride instead of the dicarboxylic compound. A limitation using this method is the yield obtained in the final step.

The members of the quinoline series were prepared by the reaction sequence shown in Scheme 2. Methyl isatin-4-carboxylate **1** reacted with anhydride acetic as the acylating agent[15] to give **4**. Then **4** underwent an intramolecular aldol condensation[15] producing the 1,2-dihydro-2-oxoquinolin-4,5-dicarboxylic acid with spontaneous dehydration to the corresponding anhydride **5**. This reacted with $POCl_3$ to give the chloro derivative **6**.[16] All efforts to dehalogenate **6** by reduction[17] proved to be unsuccessful.

The two above-mentioned syntheses started with **1**. This was prepared using an adaptation of the Sandmeyer synthesis by the reaction of methyl 3-aminobenzoate with chloral hydrate and hydroxylamine, yielding the corresponding oxyme, whose cyclization with a strong acid medium[18] produced the desired product **1**. The syntheses of the aryl- or heteroaryl-substituted naphthalic anhydrides were prepared by a Stille cross-coupling reaction (Scheme 3) by condensation of the 3- or 4-bromo-1,8-naphthalic anhydride with aryl- or heteroaryltributyltin in the presence of tetrakis-(triphenylphosphine)palladium as a catalyst in dioxane at refluxing temperature. Details of the synthesis of this series are reported elsewhere.[19] The initial 4-Br product is commercially available, and the 3-bromo-1,8-naphthalic anhydride was prepared by adding bromine to a solution of 1,8-naphthahlic anhydride in 70% nitric acid.[20] Finally, the monomeric and dimeric compounds were prepared by reaction between the corresponding anhydrides and the appropriate commercially available polyamines. The monomeric compounds contain a $(CH_2)_2NMe_2$ side chain, and the corresponding bis analogues were linked by a $(CH_2)_2NH(CH_2)_3)NH(CH_2)_2$ chain.

Biological Activity. In vitro cytotoxic potencies of the target compounds **11**−**22** and of the reference drugs amonafide and elinafide against the human colon adenocarcinoma cell line (HT29), human cervical carcinoma (HeLa), and human prostate carcinoma (PC-3) are reported in Table 1. The results indicate that all the compounds possess a good antiproliferative activity in the micromolar range, with the exception of **14** and **15**, which are the least effective in the series because the carbonyl form is the tautomeric form preferred by the quinoline chromophore (see spectral characterizations in Experimental Section). The dimeric compounds were generally more potent than the corresponding monomeric ones. Compound **19** emerges as the most potent among the new derivatives with an IC_{50} value of 0.021 μM against HT-29. This result agrees with the hypothesis that the introduction of "nonfused" tricyclic aromatic systems can enhance this activity.[9,10] Apart from **19**, a noticeable cytotoxic activity is also shown by **17** with an IC_{50} value of 0.16 μM against HT-29.

Quantitative Structure−Activity Relationship (QSAR) Analysis. The data obtained allowed us to determine quantitative structure−activity relationships.

From the data in Table 2 on HT-29 cells, we have derived eq 1:

$$p(IC_{50\ HT-29}) = 9.36\ (\pm 0.82) - 2.58\ (\pm 0.48)\ c\log P + 0.40\ (\pm 0.07)\ c\log^2 P \quad (1)$$

$$n = 9,\ s = 0.398,\ r^2 = 0.864,\ F_{2,6} = 19.07,\ \alpha < 0.005\ (S > 99.5\%)$$

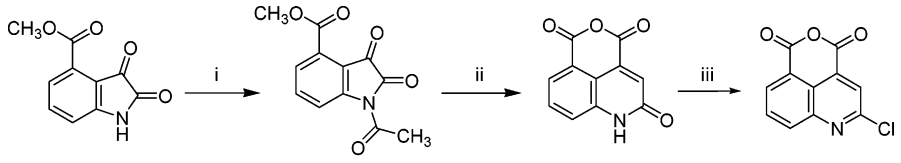
inversion point: $c\log P_0 = 3.22\ (2.97-3.52)$; outlier, **16**

where n is the number of data points, s is the standard deviation, r is the correlation coefficient, and the data within parentheses are for the 95% confidence intervals.

The predictive ability of the models was assessed for the nine compounds in the training set using the cross-validation approach and measured in terms of q^2 values:

$$q^2 = (SD - PRESS)/SD \quad (2)$$

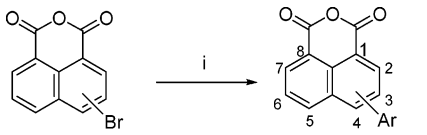
Scheme 2[a]



1 **4** **5** **6**

[a] Reagents: (i) $(CH_3CO)_2O$, reflux; (ii) aqueous NaOH, reflux; (iii) $POCl_3$, dioxane, 80−85 °C.

Scheme 3[a]



7a: 3-Br
7b: 4-Br

8: Ar = 3-phenyl
9: Ar = 3-(2-furyl)
10: Ar = 4-pheny

[a] Reagents: (i) aryltrialkyltin, $Pd(PPh_3)_4$, dioxane, reflux 48 h.

Table 2. pIC_{50} Values of Azanaphthalimide and Arylnaphthalimide Derivatives against the Human Cancer Cell Lines HT-29, HeLa, and PC-3[a]

		pIC_{50} [c]		
compd	*c* log *P* [b]	HT-29	HeLa	PC-3
11	2.39	4.93	5.47	NT
12	3.84	5.46	3.09	NT
13	3.61	4.82	5.00	NT
16[d]	1.08	5.24	5.17	4.85
17	1.22	6.80	6.57	5.27
18	3.34	5.32	5.58	5.06
19	5.75	7.68	6.64	5.96
20	2.68	5.92	6.20	5.58
21	4.42	5.44	5.82	5.19
22	3.32	5.25	5.38	4.78

[a] Compounds **14** and **15** are not included in deriving eq 1. [b] Predicted by using the PALLAS 2.0 program.[21] [c] $pIC_{50} = -\log(IC_{50})$, bearing in mind that the higher the value of pIC_{50} the more potent the compound. [d] Not included in deriving eq 1.

where the PRESS (predictive residual sum of squares) and SD (standard deviation) values are obtained as

$$\text{PRESS} = \sum(\text{property}_{\text{observed}} - \text{property}_{\text{predicted}})^2 \quad (3)$$

$$\text{SD} = \sum(\text{property}_{\text{observed}} - \text{property}_{\text{mean}})^2 \quad (4)$$

Equation 1 gives a good q^2 value of 0.864. q^2 will always be smaller than r^2. A model is considered significant[21] when $q^2 > 0.3$.

The statistics of QSAR (1) are promising, and only one compound fitted in so poorly that it had to be omitted **16**. Interestingly, such an equation shows an inverted parabola; the coefficient with the *c* log *P* term is negative and the squared term is positive. That is, as lipophilicity increases, activity first decreases to a minimum and then increases with further increment in the calculated log *P*. A constant concern in formulating a new QSAR is to find as much support as possible. In fact it is believed that a single QSAR standing alone cannot be seriously considered until one can find that it has some generality. Similar inverted parabolas have been associated with allosteric interactions for enzymes and receptors[23,24] and resistant and sensitive cancer cells.[25] The two following facts are especially noteworthy: (a) eq 1 includes two structural congeners such as the mono- and the bisnaphthalimides, with their respective bioisosteres and quinoline derivatives, and (b) the initial negative slope is rarely larger than ±1.20, suggesting something other than a simple hydrophobic interaction.[25]

It is of interest to consider the nature of the outlier. 2-Halogenated pyridines are structurally similar to the very reactive imino halides, which are the nitrogen analogues of acid chlorides.[26] The halogen atom of 2-chloropyridine is very easily displaced by nucleophilic reagents.[26] Quaternization of the quinoline ring brings about a dramatic increase in the lability of the 4-haloquinoline, and it was reported that 4,7-dichloro-1-methylquinoline methosulfate with piperidine at room temperature gives 7-chloropiperidinoquinoline.[27] It can be assumed that at physiological pH, the chlorine atom of **16** could be displaced by nucleophilic centers present in the bases of DNA, and accordingly, the anticancer activity of **16** may be the consequence of its alkylating capacity.[28]

When we tried to obtain similar QSARs for compounds shown in Table 2 with other cell lines (HeLa and PC-3), only a moderate statistical fit was achieved ($r^2 = 0.728$ and $r^2 = 0.562$, respectively), compound **16** not being included as we proceeded with eq 1. In the case of the HeLa cell line, both terms (*c* log *P* and *c* $\log^2 P$) were significant (data not shown), and this might hint that other variables need to be considered. Unfortunately, with so few points we could not attempt to use additional parameters.

In the present paper, we have a QSAR based on *c* log *P* and *c* $\log^2 P$ terms where activity against the HT-29 cell line first descends to a minimum and then begins to rise. The outlier **16** could behave as an electrophilic trap for the bases of DNA instead of an intercalator.

To elucidate whether compound **16** reacts with DNA, UV−vis spectra and ITC (isothermal titration calorimetry) thermograms were carried out. There is no evidence of reaction of compound **16** with DNA or with 2′-deoxyguanosine because the spectra of the binary mixtures is the result of the addition of the spectra of the two pure substances.

Isothermal titration calorimetry is a modern technique with wide applications to determine the binding enthalpy and heat capacity change for different compounds as DNA intercalators.[29,30] Our results confirm that there is no reaction between compound **16** and 2′-deoxyguanosine. The lack of action on DNA does not justify the above-mentioned hypothesis proposed for **16**.

The DNA binding properties of compounds **16** and **19** were also studied by viscosimetric titration with calf thymus DNA. It is known[31,32] that the DNA length increases when a drug behaves as an intercalator. The compound could be mono- or bifunctional. One method to identify the type of intercalator is by viscosimetric

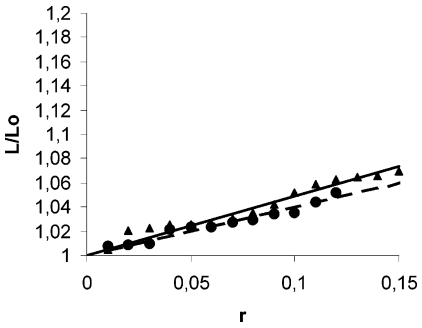


Figure 1. Relative length increase L/L_o of **16**−DNA (▲, solid lines) and **19**−DNA complexes (●, dashed lines) as a function of the molar ratio of added compound to DNA nucleotides, r.

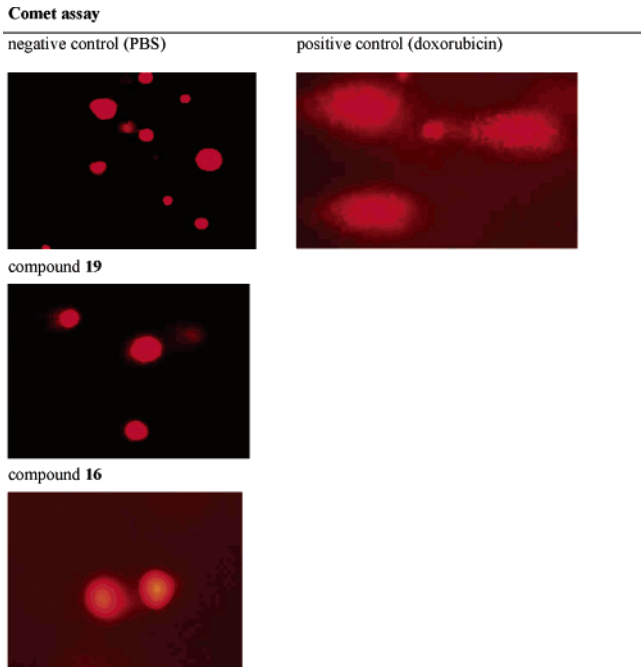


Figure 2. Tail moment for compounds **16** and **19** was 0, and the % DNA in comet was 0% (7% and 17%, respectively, for doxorubicin) in HT-29 cells.

titrations. With these measurements the relative increase in contour length, L/L_o, against r is plotted. The slope of this plot has different values depending on the functionality of the intercalator.[31,33] In Figure 1 L/L_o is plotted against r for **16** and **19**. Least-squares fitting gives a slope of 0.49 and 0.40, respectively. These values reveal that there is no evidence of intercalative binding in the viscosimetric titration assay.

Nevertheless, to further evaluate the mechanism of action, compounds **16** and **19** were selected for single-cell gel electrophoresis assay (comet assay). It is based on the property of negatively charged DNA fragments migrating when an electrical field is applied to the gel after cell lysis.[34] Doxorubicin was chosen as a positive reference, and PBS (phosphate-buffered saline, pH 7.4) was used as a negative control. One hour after treatment, the samples were observed using fluorescence. The comet assay detected no DNA damage in the cells treated with these compounds, similar to that observed in the negative control. Parent DNA damage was observed with the positive reference (Figure 2). Thus, we suggest these compounds may not express their cytotoxicity via DNA degradation.

Conclusions

The biological results obtained show that antitumor activity is not limited to those compounds possessing fused tricyclic or larger chromophores. Apart from 2-phenylnaphthalene, the most interesting chromophore appears to be 2-chloroquinoline. Both dimeric compounds with these chromophores, **17** and **19**, showed significant growth delays in vitro. The biological activity of compound **19** was encouraging for the possible further development of this class of compounds.

Some preliminary results have shown that some bisnaphthalimides with good inhibitory activity have no action against topoisomerase II.[6] The findings permit us to suspect an additional action mechanism for these kinds of compounds. Thus, the preliminary results obtained by J. C. Lacal and co-workers (personal communication) on the action of elinafide **2** on transcription factors point in this direction and could open up new perspectives. It should definitely be noted in future studies.

Experimental Section

General Methods. Melting points (uncorrected) were determined on a Stuart Scientific SMP3 apparatus. Infrared (IR) spectra were recorded with a Perkin-Elmer 1330 infrared spectrophotometer. ^{1}H and ^{13}C NMR were recorded on a Bruker 300-AC instrument. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane. Elemental analyses (C, H, N) were performed on a Perkin-Elmer 2400 CHN apparatus at the Microanalysis Service of the Complutense University, Madrid. Unless otherwise stated, all reported values are within ±0.4% of the theoretical compositions. Reactions were monitored by thin-layer chromatography (TLC) run on Merck silica gel 60 F-254 plates and iodine vapor and/or UV light detection. Flash chromatography was performed using Merck silica gel 60 (size 230−400 mesh ASTM). Unless stated otherwise, starting materials used were high-grade commercial products. The tail moment and % DNA in comet were determined by Vat-Euclid Comet Analysis Software, St. Louis, MO. 2′-Deoxyguanosine was purchased from Sigma Aldrich.

Synthetic Chemistry. Methyl 4-Isatincarboxylate (1). A solution of 13.6 g (0.09 mol) of methyl 3-aminobenzoate, 16.1 g (0.09 mol) of chloral hydrate, 234.4 g of sodium sulfate, and 19.8 g (0.29 mol) of hydroxylamine hydrochloride in 145 mL of water and 8 mL of concentrated HCl was refluxed for 2 h and then kept at room temperature overnight. The cream-colored isonitroso intermediate was filtered off, washed with water, and dried (16.0 g, 80%). This compound, 16.0 g (0.07 mol), was added in portions, while stirring over 30 min, to concentrated H_2SO_4 (70 mL) maintained at 70−75 °C. The mixture was then heated at 80 °C until the reaction was complete (TLC, $CHCl_3$/EtOH, 8:2) and poured onto ice. The aqueous phase was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness to yield the isatin derivative, typically as an orange solid, which was purified by gradient flash column chromatography on silica gel, eluting with $CHCl_3$/EtOH, 8:2, to yield the title compound (5 g, 34%), mp 215−217 °C. IR (KBr): 3250, 1765, 1740, 1720, 1200 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 3.92 (s, 3H, OCH_3), 7.07 (d, 1H, ArH), 7.22 (d, 1H, ArH), 7.66 (t, 1H, ArH), 11.20 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 52.60, 115.42, 123.21, 129.60, 131.61, 138.05, 151.52, 158.56, 166.75, 181.93. Anal. ($C_{10}H_7NO_4$) C, H, N.

Preparation of 2-Aryl-4,5-quinolinedicarboxylic Anhydrides 2 and 3. General Procedure for Pfitzinger Reaction. A mixture of methyl 4-isatincarboxylate (2.5 mmol), ethanol (5 mL), and 85% potassium hydroxide (7.3 mmol) was stirred at room temperature for 30 min. The appropriate ketone (1.7 mmol), dissolved in EtOH (1 mL), was added, and the reaction was stirred at room temperature for 1 h and at

80 °C for 72 h. Evaporation of the solvent produced a residue that was dissolved in H_2O (2 mL), and the solution was washed twice with Et_2O (5 mL). The ice-cold aqueous phase was acidified to pH 1 with 37% HCl, and the precipitate was collected by suction filtration, washed with H_2O, and dried. Typically, yields of this reaction were very poor. The following were prepared in this manner.

2-Phenyl-4,5-quinolinedicarboxylic Anhydride (2). The precipitate formed was filtered and dried to give **2** as a light-cream solid (22%), mp 198−200 °C. IR (KBr): 1780, 1750 cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.59 (m, 3H, ArH), 7.71 (t, 1H, ArH), 7.86 (t, 1H, ArH), 8.19 (d, 1H, ArH), 8.29 (d, 1H, ArH), 8.46 (s, 1H, ArH), 8.65 (d, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 128.74, 130.01, 136.93, 138.65, 138.79, 138.89, 139.87, 140.70, 142.60, 147.03, 150.14, 158.08, 165.77, 177.50, 178.43. Anal. ($C_{17}H_9NO_3$) C, H, N.

2-(2′-Naphthyl)-4,5-quinolinedicarboxylic Anhydride (3). The precipitate formed was filtered and dried to give **3** as a light-cream solid (15%), mp 218−220 °C. IR (KBr): 1760, 1730 cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.60 (m, 4H, ArH), 7.79 (t, 1H, ArH), 7.99 (m, 3H, ArH), 8.10 (d, 1H, ArH), 8.32 (d, 1H, ArH), 8.35 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 118.51, 122.93, 123.82, 124.50, 125.91, 125.94, 126.05, 126.20, 128.01, 128.09, 128.51, 130.52, 132.11, 132.96, 134.13, 137.10, 149.95, 154.63, 166.00, 177.03, 179.02. Anal. ($C_{21}H_{11}NO_3$) C, H, N.

2-Oxo-1,2-dihydro-4,5-quinolinedicarboxylic Anhydride (5). *N*-Acetylisatin derivative **4** was prepared by refluxing 1.5 g of **1** (7.3 mmol) in 3.5 mL of anhydride acetic for 2 h (TLC, ethyl acetate/hexane, 11:1). The solvent was removed to dryness to obtain a crude that was purified by gradient flash column chromatography on silica gel, eluting with ethyl acetate/hexane, 11:1, 1 g (55%), mp 121−123 °C. IR (KBr): 1750, 1720, 1705, 1670 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.75 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 7.63 (d, 1H, ArH), 7.76 (t, 1H, ArH), 8.62 (d, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 24.28, 53.16, 116.55, 120.73, 126.61, 130.68, 138.04, 148.92, 156.66, 165.24, 168.61, 177.41. Anal. ($C_{12}H_9NO_5$) C, H, N. Compound **4**, 1 g (4 mmol), was added, with stirring, to NaOH solution (8 mmol) at 90 °C for 1 h. The pH was taken to 2 with concentrated HCl to give a white solid, which was collected by suction filtration to yield **5** (0.82 g, 93%), mp >250 °C; IR (KBr): 3450, 1760, 1700, 1650 cm^{-1}. ^1H NMR (DMSO-d_6): δ 6.77 (s, 1H, ArH), 7.54 (m, 3H, ArH), 12.18 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 113.30, 118.97, 123.39, 123.70, 130.09, 131.91, 140.01, 140.28, 160.65, 167.36, 168.70. Anal. ($C_{11}H_5NO_4$) C, H, N.

2-Chloro-4,5-quinolinedicarboxylic Anhydride (6). A mixture of 0.1 g (0.5 mmol) of **5** and 0.06 mL (0.064 mmol) of $POCl_3$ in 1,4-dioxane (3 mL) was maintained at 80−85 °C while being stirred for about 15 min until most of the solid had dissolved and was then warmed for an additional 15 min until dissolution was complete. The unreacted $POCl_3$ was then removed under reduced pressure. Ice was added to the residue, and the precipitate that separated was filtered and washed with water to give **6** as a yellow solid (0.047 g, 40%), mp >250 °C. IR (KBr): 1782, 1760 cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.18 (t, 1H, ArH), 8.37 (s, 1H, ArH), 8.51 (d, 1H, ArH), 8.57 (d, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 115.50, 119.02, 122.11, 123.90, 131.11, 131.92, 133.01, 141.02, 149.40, 167.40, 169.70. Anal. ($C_{11}H_4ClNO_3$) C, H, Cl, N.

Preparation of 3- or 4-Aryl and Heteroaryl-1,8-Naphthalic Anhydrides (8−10). General Procedure for Stille Coupling Reaction. A mixture of the bromo-1,8-naphthalic anhydride derivative (12.6 mmol), aryl or heteroaryltributiltin derivative (15.3 mmol), and tetrakis(triphenylphosphine)-palladium(0) (0.41 mmol) in 50 mL of dioxane was heated to reflux for 48 h. After the mixture was cooled to room temperature, the solvent was evaporated to dryness. The following anhydrides were prepared in a similar manner.

3-Phenylnaphthalic Anhydride (8). This was prepared from 3-bromonaphthalic anhydride **7a**.[20] The residue was purified by gradient flash column chromatography on silica gel, eluting with (CH_2Cl_2/hexane, 8:2) to yield the corresponding anhydride (84%), mp >250 °C. IR (KBr): 1775 1740 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.56 (m, 2H, ArH), 7.80 (m, 3H, ArH), 8.31 (d, 1H, ArH), 8.34 (d, 1H, ArH) 8.48 (s, 1H, ArH), 8.62 (t, 1H, ArH), 8.88 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 118.66, 119.24, 127.01, 127.39, 127.41, 127.97, 129.27, 132.41, 132.99, 133.35, 135.27, 135.37, 138.31, 140.71, 160.38. Anal. ($C_{18}H_{10}O_3$) C, H, N.

3-(2′-Furyl)naphthalic Anhydride (9). This was prepared from 3-bromonaphthalic anhydride **7b**.[20] The residue was purified by gradient flash column chromatography on silica gel, eluting with (CH_2Cl_2/hexane, 7:3) to yield the corresponding anhydride (80%), mp 188−190 °C. IR (KBr): 1780, 1740 cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.60 (t, 1H, ArH), 6.97 (d, 1H, ArH), 7.61 (s, 1H, ArH), 7.81 (t, 1H, ArH) 8.31 (d, 1H, ArH), 8.54 (d, 1H, ArH), 8.57 (d, 1H, ArH), 8.87 (s, 1H, ArH). Anal. ($C_{16}H_8O_4$) C, H, N.

4-Phenylnaphthalic Anhydride (10). This was prepared from 4-bromonaphthalic anhydride **7b**. The residue was purified by gradient flash column chromatography on silica gel, eluting with (CH_2Cl_2/hexane, 8:2) to yield the corresponding anhydride (89%), mp >190−200 °C. IR (KBr): 1770, 1749 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.52 (m, 5H, ArH), 7.74 (d, 1H, ArH), 7.79 (t, 1H, ArH), 8.39 (d, 1H, ArH), 8.65 (d, 1H, ArH), 8.68 (d, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 117.67, 118.95, 127,32, 128.32, 128.84, 128.93, 129.72, 130.30, 133.05, 133.40, 134.20, 148.55, 160.40. Anal. ($C_{18}H_{10}O_3$) C, H, N.

General Procedure for the Preparation of Monomeric Compounds. A mixture of the corresponding anhydride (2.0 mmol) and *N*,*N*-dimethylethylenediamine (2.0 mmol) in 10 mL of DMF (for quinoline derivatives) or $CHCl_3$ (for naphthalene derivatives) was heated to reflux for 24 h. The solvent in the reaction mixture was evaporated.

***N*,*N*-Dimethyl-*N*′-2-{5-phenyl-1,3,-dioxo-1,3-dihydropyrido[3,4,5-*de*]quinolin-2-yl}ethanamine (11).** This was prepared from **2**. The residue was purified by gradient flash column chromatography on silica gel, eluting with ($CHCl_3$/EtOH, 10:1) to yield the corresponding monointercalator as a free base (34%), mp >250 °C. IR (KBr): 1720, 1680 cm^{-1}. ^1H NMR (CD_3OD): δ 2.36 (s, 6H, CH_3), 2.71 (t, 2H, CH_2), 4.28 (t, 2H, CH_2), 7.54 (m, 5H, ArH), 7.97 (m, 1H, ArH), 8.18 (m, 1H, ArH), 8.41 (m, 1H, ArH), 8.62 (m, 1H, ArH). ^{13}C NMR (CD_3OD): δ 41.10, 43.92, 57.30, 117.71, 125.20, 127.11, 127.14, 127.19, 129.00, 131.33, 131.72, 134.51, 138.45, 138.80, 139.70, 145.37, 159.79, 175.91, 171.01. Anal. ($C_{21}H_{19}N_3O_2$) C, H, N.

***N*,*N*-Dimethyl-*N*′-2-{5-(2′-naphthyl)-1,3,-dioxo-1,3-dihydropyrido[3,4,5-*de*]quinolin-2-yl}ethanamine (13).** This was prepared from **3** in 24% yield, mp 246−248 °C. IR (KBr): 1719, 1675 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.28 (s, 6H, CH_3), 2.59 (t, 2H, CH_2), 4.23 (t, 2H, CH_2), 7.44 (m, 2H, ArH), 7.82 (m, 5H, ArH), 8.24 (d, 1H, ArH), 8.37 (t, 1H, ArH), 8.49 (s, 1H, ArH), 8.71 (s, 1H, ArH). Anal. ($C_{25}H_{21}N_3O_2$) C, H, N.

***N*,*N*-Dimethyl-*N*′-{2-[1,3,5-trioxo-1,3,5,6-tetrahydropyrido[3,4,5-*de*]quinolin-2-yl}ethanamine (14).** This was prepared from **5**. The residue was purified by gradient flash column chromatography on silica gel, eluting with ($CHCl_3$/EtOH, 10:1) to yield the corresponding monointercalator as a free base (63%), mp 210−218 °C. IR (KBr): 3410, 1670, 1650, 1620 cm^{-1}. ^1H NMR (D_2O): δ 2.52 (s, 6H, CH_3), 2.98 (t, 2H, CH_2), 3.97 (t, 2H, CH_2), 6.01 (s, 1H, ArH), 7.10 (d, 1H, ArH), 7.29 (t, 1H, ArH), 7.53 (d, 1H, ArH). Anal. ($C_{15}H_{15}N_3O_3$) C, H, N.

***N*,*N*-Dimethyl-*N*′-2-{5-chloro-1,3,-dioxo-1,3-dihydropyrido[3,4,5-*de*]quinolin-2-yl}ethanamine (16).** This was prepared from **6**. The residue was purified by gradient flash column chromatography on silica gel, eluting with ($CHCl_3$/EtOH, 10:1) to yield the corresponding monointercalator as a free base (60%), mp 238−240 °C. IR (KBr): 1720, 1675 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.93 (s, 6H, CH_3), 3.48 (t, 2H, CH_2), 4.42 (t, 2H, CH_2), 8.18 (t, 1H, ArH), 8.30 (s, 1H, ArH), 8.45 (d, 1H, ArH), 8.58 (d, 1H, ArH). Anal. ($C_{15}H_{14}ClN_3O_2$) C, H, Cl, N.

***N*,*N*-Dimethyl-*N*-{2-[1,3-dioxo-5-phenyl-2,3-dihydro-1*H*-benzo[*de*]isoquinol-5-yl)ethyl}ethanamine (18).** This was prepared from **8**. The residue was purified by gradient flash column chromatography on silica gel, eluting with ($CHCl_3$/EtOH/NH_3, 10:1:0.1%) to yield the corresponding

monointercalator as a free base (60%), mp 150−154 °C. IR (KBr): 1700, 1655 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.36 (s, 6H, CH_3), 2.67 (t, 2H, CH_2), 4.34 (t, 2H, CH_2), 7.48 (m, 2H, ArH), 7.52 (m, 4H, ArH), 8.18 (d, 1H, ArH), 8.31 (s, 1H, ArH), 8.52 (d, 1H, ArH), 8.80 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 38.11, 45.70, 56.92, 122.41, 122.95, 127.10, 127.27, 128.26, 129.01, 130.55, 131.03, 131.94, 133.90, 138.99, 139.81, 167.40. Anal. ($C_{22}H_{20}N_2O_2$) C, H, N.

***N,N*-Dimethyl-*N*-{2-[1,3-dioxo-5-(2-furyl)-2,3-dihydro-1*H*-benzo[*de*]isoquinol-5-yl)ethyl}ethanamine (20).** This was prepared from **9**. The residue was purified by gradient flash column chromatography on silica gel, eluting with ($CHCl_3$/EtOH, 8:2) to yield the corresponding anhydride (87%), mp 130−132 °C. IR (KBr): 1700, 1655 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.51 (s, 6H, CH_3), 2.90 (t, 2H, CH_2), 4.34 (t, 2H, CH_2), 6.56 (s, 1H, ArH), 7.26 (m, 4H, ArH), 7.70 (t, 1H, ArH), 8.12 (d, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 38.12, 45.71, 56.91, 107.41, 112.18, 122.48, 122.92, 126.96, 127.05, 129.40, 131.86, 133.85, 143.24, 152.07, 163.97. Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

***N,N*-Dimethyl-*N*-{2-[1,3-dioxo-6-phenyl-2,3-dihydro-1*H*-benzo[*de*]isoquinol-5-yl)ethyl}ethanamine (22).** This was prepared from **10**. The residue was purified by gradient flash column chromatography on silica gel, eluting with ($CHCl_3$/EtOH, 10:1) to yield the corresponding anhydride (90%), mp 188−190 °C. IR (KBr): 1710, 1655 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.36 (s, 6H, CH_3), 2.67 (t, 2H, CH_2), 4.32 (t, 2H, CH_2), 7.48 (m, 2H, ArH), 7.52 (m, 4H, ArH), 8.18 (d, 1H, ArH), 8.31 (s, 1H, ArH), 8.52 (d, 1H, ArH), 8.80 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 38.12, 45.72, 56.91, 121.65, 122.77, 126.76, 127.78, 128.61, 129.82, 130.81, 131.16, 132.62, 138.74, 146.86, 164.10. Anal. ($C_{22}H_{20}N_2O_2$) C, H, N.

General Procedure for the Preparation of Dimeric Compounds. A mixture of the corresponding anhydride (2.0 mmol) and *N*,*N*-bis(2-aminoethyl)-1,3-propanediamine (1.0 mmol) in 10 mL of DMF (for quinoline derivatives) or $CHCl_3$ (for naphthalene derivatives) was heated to reflux for 24 h. The solvent in the reaction mixture was evaporated. The remaining residue was flash-chromatographed to provide the bis intercalator as a free base.

***N,N′*-Bis{2-[5-phenyl-1,3-dioxo-1,3-dihydro-2*H*-pyrido-[3,4,5-*de*]quinolin-2-yl]ethyl}propylenediamine (12).** This was prepared from **2** in 32% yield, mp 240−244 °C. IR (KBr): 1710, 1675 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.67 (q, 2H, CH_2), 2.74 (t, 4H, CH_2), 2.92 (t, 4H, CH_2), 4.18 (t, 4H, CH_2), 7.42 (m, 10H, ArH), 7.82 (t, 2H, ArH), 8.12 (d, 2H, ArH), 8.37 (dd, 2H, ArH), 8.60 (s, 2H, ArH). Anal. ($C_{41}H_{34}N_6O_4$) C, H, N.

***N,N′*-Bis{2-[1,3,5-trioxo-1,3,5,6-tetrahydropyrido[3,4,5-*de*]quinolin-2-yl]ethyl}propylenediamine (15).** This was prepared from **5** in 35% yield, mp 242−244 °C. IR (KBr): 3410, 1710, 1680, 1650 cm^{-1}. ^1H NMR (DMSO-d_6 + TFA): δ 2.07 (q, 2H, CH_2), 3.05 (t, 4H, CH_2), 3.10 (dd, 4H, CH_2), 3.21 (t, 4H, CH_2), 6.95 (s, 2H, ArH), 7.60 (m, 6H, ArH). Anal. ($C_{29}H_{26}N_6O_6$) C, H, N.

***N,N′*-Bis{2-[5-chloro-1,3-dioxo-1,3-dihydro-2*H*-pyrido-[3,4,5-*de*]quinolin-2-yl]ethyl}propylenediamine (17).** This was prepared from **6** in 28% yield, mp 222−225 °C. IR (KBr): 1720, 1670 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.25 (q, 2H, CH_2), 3.07 (t, 4H, CH_2), 3.33 (t, 4H, CH_2), 4.38 (t, 4H, CH_2), 8.20 (m, 2H, ArH), 8.42 (s, 2H, ArH), 8.60 (m, 4H, ArH). Anal. ($C_{29}H_{24}Cl_2N_4O_4$) C, H, Cl, N.

***N,N′*-Bis{2-[5-phenyl-1,3-dioxo-2,3-dihydro-1*H*-benzo-[*de*]isoquinol-5-yl]ethyl}-1,3-propanodiamine (19).** This was prepared from **8** in 62% yield, mp 218−220 °C. IR (KBr): 1720, 1670 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.77 (q, 2H, CH_2), 2.84 (t, 4H, CH_2), 3.01 (t, 4H, CH_2), 4.27 (t, 4H, CH_2), 7.47 (m, 4H, ArH), 7.64 (s, 6H, ArH), 8.06 (m, 3H, ArH), 8.09 (d, 2H, ArH), 8.21 (dd, 3H, ArH), 8.44 (d, 2H, ArH). ^{13}C NMR ($CDCl_3$): δ 28.54, 39.32, 47.49, 48.07, 77.21, 122.41, 122.95, 126.72, 127.11, 128.23, 129.01, 130.79, 131.05, 131.90, 133.79, 138.81, 139.67, 164.17. Anal. ($C_{43}H_{36}N_4O_4$) C, H, N.

***N,N′*-Bis{2-[5-(2-furyl)-1,3-dioxo-2,3-dihydro-1*H*-benzo-[*de*]isoquinol-5-yl]ethyl}-1,3-propanodiamine (21).** This was prepared from **9** in 62% yield, mp 222−225 °C. IR (KBr): 1700, 1655 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.13 (q, 2H, CH_2), 3.13 (t, 4H, CH_2), 3.23 (t, 4H, CH_2), 4.30 (t, 4H, CH_2), 6.56 (s, 2H, ArH), 6.86 (s, 2H, ArH), 7.26 (m, 2H, ArH), 7.57 (dd, 2H, ArH), 7.62 (d, 2H, ArH), 8.11 (d, 2H, ArH), 8.35 (d,2 H, ArH), 8.55 (s, 2H, ArH). ^{13}C NMR ($CDCl_3$): δ 28.76, 37.55, 46.81, 47.86, 77.01, 106.70, 11.32, 121.01, 121.37, 125.71, 126.43, 128.40, 129.71, 130.63, 133.79, 142.39, 150.87, 163.33. Anal. ($C_{39}H_{32}N_4O_6$) C, H, N.

In Vitro Cytotoxicity Assays. The cell lines used were human colon carcinoma (HT-29) (ATCC, HTB 38), human cervical carcinoma (HeLa) (ATCC, CCL 2), and human prostate carcinoma (PC-3) (ECACC, 90112714). For each experiment cultures were seeded from frozen stocks. Each cell line was maintained in its appropriate medium and incubated at 37 °C in a 5% CO_2 atmosphere.

All cell lines were in the logarithmic phase of growth when the assay of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was carried out. Cells were harvested and seeded into 96-well tissue culture plates at a density of 2.5×10^4 cells/well in 150 μL aliquots of medium. The concentrations tested were serial dilutions of a stock solution (1×10^{-5} M in DMSO) with phosphate-buffered saline (PBS) and were added 24 h later. The assay was ended after 72 h of drug exposure, and PBS was used as a negative control and doxorubicin as a positive control.

After a 72 h exposure period, cells were washed twice with PBS (phosphate-buffered saline, pH 7.4), and then 50 μL/well of MTT reagent (1 mg/mL in PBS; Sigma) and 150 μL/well of prewarmed medium were added. The plates were returned to the incubator for 4 h. Subsequently, DMSO was added as solvent. Absorbance was determined at 570 nm with a microplate reader (Opsys MR). All experiments were performed at least three times, and the average of the percentage absorbance was plotted against concentration. The concentration of drug required to inhibit 50% of cell growth (IC_{50}) was then calculated for each compound.

Alkaline Single-Cell Gel Electrophoresis Assay. The alkaline single-cell gel electrophoresis assay (comet assay) detects DNA damage in individual cells embedded in agarose. The test was performed on HT-29 cells following the method described by Moinet-Hedin et al.[34] After 1 h of treatment with the drug, cells were centrifuged and resuspended in low-melting-point (LMP) agarose at 37 °C. Doxorubicin was chosen as a positive reference, and PBS was used as a negative control. The cell suspension was put on a slide precoated with normal agarose and a glass cover slip was added. After solidification at 0 °C, the glass cover slip was gently removed and a third layer of 0.5% of LMP agarose in PBS was added and run for solidification. The slides were put in a lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris, pH 10, with freshly added 1% Triton X-100 and 10% DMSO) for 1 h and were rinsed in the electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH 13) for 40 min. Electrophoresis (300 mA, 0.7 V/cm) was then performed for 24 min in fresh buffer. The slides were washed twice in neutralization buffer (0.4 M Tris, pH 7.5) and stained with ethidium bromide (20 μg/mL). They were observed using a fluorescence microscope (Nikon) with an excitation filter of 515−560 nm and a barrier filter of 580 nm.

Isothermal Titration Calorimetry. Experiments were carried out at 37 °C using a Microcal VP-ITC microcalorimeter (Microcal Inc., Northampton, MA). The Origin software (Microcal) was used for data acquisition and analysis. 2′-Deoxyguanosine, 0.057 mM, in Tris buffer (50 mM, pH 6.9) was titrated using a 2.16 mM compound **16** solution in the same buffer (30 injections of 3 μL each), using a 250 μL syringe rotating at 450 rpm. The injection time was 6 s, and the delay between injections was 4 min. The peaks produced during titration were converted to heat output per injection by integration and correction for the cell volume and sample concentration.

UV−Visible Spectra. For spectrometric determinations we have used a spectrophotometer Perkin-Elmer Lambda 14 with a digital temperature controller PTP-6. The spectra of com-

pound **16**, 2′-deoxyguanosine, sonicated DNA, **16**-sonicated DNA, and **16**-2′-deoxyguanosine have been registered at 37 °C.

DNA. Calf thymus DNA was purchased from Sigma Chemical Co. as the highly polymerized sodium salt. For viscosimetric experiments the DNA has been sonicated to fragments of approximately 4.5×10^5 Da determined as described by Eigner and Doty.[35]

The sonicated DNA sample displayed an A_{260}/A_{280} ratio of 1.92. This spectral result is consistent with published values.[36]

Viscosimetric Titrations. The viscosimetric measurements were performed in an Ubbelohde microviscometer at 25 ± 0.05 °C. Solutions of sonicated DNA and the selected compound have been prepared in Tris buffer (50 mM, pH 6.9). These solutions have a different molar ratio, *r*, of added compound to DNA nucleotides. Flow times were measured with a Schott-Geräte Viscosystem AVS 350 to an accuracy of 0.01%. Time readings were recorded in triplicate to 0.01 s.

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