Synthesis and In Vitro Antitumor Activity of Novel Ring D Analogues of the Marine Pyridoacridine Ascididemin: Structure−Activity Relationship

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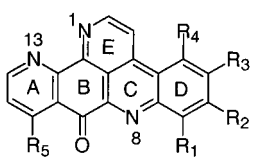
Marine compounds with pyridoacridine skeletons are known to exhibit interesting antitumor activities. Ascididemin has already been reported as displaying significant antitumor activities in vitro and has also been found to have a relatively high global toxicity in vivo. We synthesized a series of 16 analogues (among which 11 compounds were different from previously described ones) with the aim of developing new anticancer agents with significantly improved efficacy/tolerability ratios. These compounds were obtained either by total synthesis from 5,8-quinolinedione and substituted 2-aminoacetophenones or by the direct substitution of ascididemin. The different compounds and ascididemin used as the control compound were tested at six different concentrations on 12 different human cancer cell lines of various histopathological types (glioblastomas and breast, colon, lung, prostate, and bladder cancers). The IC_{50} value (i.e., the drug concentration inhibiting the mean growth value of the 12 cell lines by 50%) of these compounds ranged over five log concentrations, i.e., between 10 000 and 0.1 nM. For several new chemical entities, the antitumor activity (determined in vitro) and tolerability (determined in vivo) were superior to those of the parent alkaloids, i.e., ascididemin and 2-bromoleptoclinidone.

Introduction

Researchers have long been investigating natural products in the quest for new anticancer drugs. Marine organisms have provided a large number of new compounds including bryostatin,[1] dolastatin,[2] cryptophycin,[3] aplidine,[4] and ecteinascidin 743,[5] all of which are currently undergoing clinical trials.

To date, the largest family of marine alkaloids characterized has been based upon the pyrido[2,3,4-*kl*]-acridine skeleton.[6] Ascididemin **1**, isolated by Kobayashi et al.[7] from the ascidian *Didemnum sp.*, is one of the first examples of these compounds. Some substituted ascididemins such as bromoleptoclinidone **2**,[8] 11-hydroxyascididemin **3**,[9] and neocalliactine **4**[10] were also isolated from marine sources, whereas 11-methoxyascididemin **5**,[11] 1- and 3-nitroascididemins (**6** and **7**),[12] and ring E-substituted ascididemins[13] were obtained by total synthesis. Bracher[14] and Kitahara et al.[11,15] have described 3-methoxyascididemin **16** as an intermediate product in two separate total synthesis processes of neocalliactine **4** (Figure 1).

It emerges from all of these published data that these different compounds have cytotoxic properties with an IC_{50} value in the micromolar range and that the most active of them remains ascididemin itself. In a recent paper, Copp and co-workers[16] have shown that **1** can act to damage DNA oxidatively via a thiol-dependent conversion of oxygen to DNA-cleaving radicals. In view of this original mode of action, ascididemin analogues appear therefore to have good prospects as anticancer drug candidates. As part of our work related to marine alkaloid analogues,[17] we are specifically interested in novel ring D-substituted ascididemins, and in the present paper, we describe the synthesis of these compounds and their in vitro antitumor activity and discuss the influence of the different substituents on this activity.



1, $R_1 = R_2 = R_3 = R_4 = R_5 = H$
2, $R_2 = Br$; $R_1 = R_3 = R_4 = R_5 = H$
3, $R_5 = OH$; $R_1 = R_2 = R_3 = R_4 = H$
4, $R_3 = OH$; $R_1 = R_2 = R_4 = R_5 = H$
5, $R_5 = OMe$; $R_1 = R_2 = R_3 = R_4 = H$
6, $R_1 = NO_2$; $R_2 = R_3 = R_4 = R_5 = H$
7, $R_3 = NO_2$; $R_1 = R_2 = R_4 = R_5 = H$

Figure 1. (a) Numbered according to IUPAC nomenclature rules. (b) Compounds **6** and **7**, described in Gellerman's paper (ref 12) as 1-nitroascididemin and 3-nitroascididemin, are, respectively, 7-nitroascididemin and 5-nitroascididemin according to IUPAC nomenclature rules.

Chemistry

The different ascididemin analogues (Table 1) were synthesized according to two main pathways: (i) substitution of ascididemin **1** for compounds **8**−**13** and **19** and (ii) total synthesis using Bracher's methodology[18] for the other derivatives. In addition, a scenario for the design of difunctional compounds was also evaluated (through the preparation of compound **19**).

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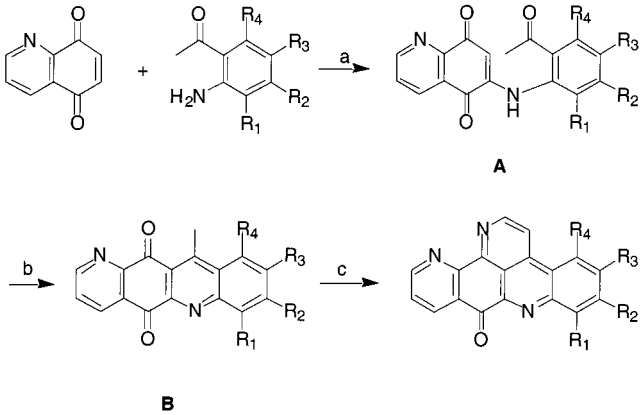
Table 1[a]

	R_1	R_3	R_4		R_1	R_3	R_4
8	NH_2			**14**		Cl	
9		Br		**15**		Me	
10		NH_2		**16**		OMe	
11		$NHCH_2CH_2Cl$		**17**		NMe_2	
12		$N(CH_2CH_2Cl)_2$		**18**		NHBn	
13		$NHCH_2CH_2NMe_2$		**19**		NH_2	Br

[a] R_2 and R_5 = H.

Scheme 1[a]



[a] Reagents: (a) $CeCl_3 \cdot 7H_2O$, EtOH, room temperature. (b) Concentrated H_2SO_4/AcOH, reflux. (c) DMF-DEA, DMF, 120 °C. (d) NH_4Cl, AcOH or EtOH, reflux.

The nitration and bromination reactions of **1** gave only substitutions on the benzenic ring at two different positions, R_1 for NO_2 and R_3 for Br. The nitration of **1** with a mixture of fuming HNO_3/concentrated H_2SO_4 (130 °C, 1.5 h) afforded compound **6** exclusively. In contrast to what was reported by Kashman,[12] we observed no substitution at R_3 (formation of compound **7**). The iron reduction of the nitro group of **6** in acetic acid media gave the corresponding amino-derivative **8**. The direct bromination of **1** led to the compound substituted at R_3 (**9**). The treatment of **9** by sodium azide in dimethylformamide (DMF) directly gave the amino-derivative **10**. Such a spontaneous reduction had already been observed by Kubo and co-workers[19] in the synthesis of cystodamine. The reductive aminoalkylation of compound **10** with chloroethanal allowed both the mono- and the disubstitution of the amine and so provided access to substituted N-derivatives **11** and **12**, respectively. The monosubstituted compound **13** was obtained when the aldehyde was dimethyl aminoethanal.

Bracher's methodology, which is the more generally applicable procedure reported to date for the synthesis of ascididemin derivatives, starts by the condensation of quinoline-5,8-dione with an ortho-aminoacetophenone, as indicated in Scheme 1. After the acid-catalyzed cyclization of the condensed product, the final ring E annulation is achieved by using DMF diethylacetal (DMF-DEA) in DMF under nitrogen to form an enamine, which is then cyclized into the pentacyclic product with ammonium chloride in ethanol or acetic acid.

The different precursors (which are substituted aminoacetophenone compounds) of compounds **14**−**18** were synthesized according to previously described procedures (see Experimental Section). 10-Methoxyascididemin **5** was prepared according to Kitahara et al.'s procedure.[15] We are also interested in an attempt to design difunctional compounds. Compound **19** disubstituted on ring D was thus obtained by the bromination of compound **10**.

Pharmacology

In Vitro Determination of the Drug-Induced Inhibition of Human Cancer Cell Line Growth. For each of the 17 compounds under study (including ascididemin), six concentrations were tested on 12 different human cancer cell lines including various histopathological types (glioblastomas and breast, colon, lung, prostate, and bladder cancers). We made use of the colorimetric 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which indirectly assesses the effect of potentially anticancer compounds on the overall growth of adherent cell lines.[20] The IC_{50} values, i.e., the concentration that reduced the mean growth value of the 12 cell lines by 50%, was determined for each drug, in comparison with the mean control growth value. Table 2 illustrates the individual IC_{50} values of the different compounds obtained for each of the 12 cell lines under study; the in vivo global toxic effects as revealed by the maximum tolerated dose (MTD) index are reported in Table 3.

These data show that the IC_{50} values range over five logarithmic concentrations. As indicated by the values of the mean IC_{50}, compounds **6**, **8**, **10**, **11**, and **17** exhibited significantly greater in vitro antitumor activity (range, 7−53 nM) than the natural substance ascididemin **1** (mean IC_{50} = 100 nM) as far as the overall panel of solid tumors (n = 12) is concerned. However, compounds **9**, **13**, **15**, and **16** selectively exhibited higher cytotoxic potency than natural ascididemin against cell lines (such as bladder, colon, breast, lung, and glioblastoma cancer cell lines). Besides, the in vitro antitumor activities of compounds **2**, **12**, **18**, and **19** are comparable to that of ascididemin (compound **1**), again with some degree of specificity (i.e., compounds **12**, **18**, and **19**). More interestingly, even though the 11 new ascididemin derivatives exhibited more potent in vitro cytotoxic activity than the natural compounds (**1** and **2**) on a majority of cancer cell lines, their systemic in vivo toxicity (defined by the MTD in vivo index) was not simultaneously increased. In some cases, this toxicity even dramatically decreased. Indeed, except for compounds **6**, **13**, and **15**, which showed similar systemic toxicity (MTD = 20 mg/kg) as ascididemin, the remaining compounds exhibited an improvement (at least by a factor of 8) in the in vivo tolerability (MTD > 160 mg/kg). However, while the improvement of in vitro cytotoxic potency was associated with a lower acute in vivo toxicity for a significant number of compounds from the present study, no clear-cut relationship emerged for those two properties in the case of compounds **6** and **15** when compared to the others.

Discussion

Ascididemin **1** and its natural analogues **2** and **3** have been tested at the U.S. National Cancer Institute (NCI) at the stage of primary in vitro screening. Comprehensive testing has demonstrated that all of the compounds exhibit selective cytotoxicity against certain cancer types

Table 2. Characterization of the In Vitro Cytotoxic-Related Antitumor Effects (IC_{50} Value in nM) of the Compounds Listed in Table 1[a]

	cell lines											
compds	U-87MG	U-373MG	SW1088	T24	J82	HCT-15	LoVo	MCF7	T-47D	A549	A-427	PC-3
1	70	500	600	800	300	6	900	70	600	200	60	8
2	500	600	400	60	500	100	600	90	60	400	60	80
3	4000	6000	6000	4000	6000	4000	5000	2000	3000	5000	1000	5000
4	not available for testing											
5	500	800	2000	600	2000	500	600	600	900	600	600	900
6	4	7	6	5	20	40	500	5	400	500	8	200
7	not available for testing											
8	300	50	80	6	100	30	3000	5	400	90	10	400
9	500	200	500	40	400	60	600	50	50	500	50	60
10	40	8	80	7	50	40	2000	20	70	8	5	300
11	6	2	0,9	1	9	7	90	10	2000	10	0,9	80
12	40	60	50	40	90	70	4000	300	700	60	20	500
13	30	70	500	10	90	80	2000	30	2000	400	10	80
14	400	600	900	90	500	80	800	500	400	700	400	300
15	60	100	400	60	400	60	500	30	40	100	50	60
16	70	80	300	60	90	50	900	80	600	80	100	80
17	30	50	200	20	60	60	400	6	80	90	9	100
18	70	90	700	50	400	50	900	40	800	60	400	800
19	80	10	200	70	200	300	4000	70	1000	100	8	500

[a] The IC_{50} value constitutes the concentration of the compound that inhibits the growth of the human cancer cells by 50% as compared to the control value. Results are reported as mean values ($n = 3$). The values for the standard errors are not reported here (for the sake in clarity of the table) because they reached less than 3% of the mean values. Six concentrations ranging from 10 000 to 0.1 nM were assayed on 12 different human cancer cell lines for each compound under study. The drug-induced effects at cell line growth level were determined by means of the MTT colorimetric assay.

Table 3. Mean Cytotoxic Potency (In Vitro/12 Cell Lines) IC_{50} (nM)[a] and MTD[b] (mg/kg) in Mice

compds	mean IC_{50}[a] (nM)	MTD[b] (mg/kg)	compds	mean IC_{50}[a] (nM)	MTD[b] (mg/kg)
1	100	20	**12**	100	>160
2	120	40	**13**	60	40
3	3200	>160	**14**	270	>160
5	480	>160	**15**	60	20
6	10	20	**16**	90	>160
8	53	>160	**17**	37	>160
9	80	>160	**18**	140	>160
10	21	>160	**19**	140	>160
11	7	>160			

[a] Mean IC_{50} was established as the arithmetic mean of the 12 IC_{50} values reported in Table 2. [b] MTD was defined following single ip injection to B6D2F1/jico mice.

in vitro. In the present work, we show that these three compounds have rather different levels of in vivo toxicity (in the 20−160 mg/kg/day dose range). Among these natural compounds, 11-hydroxyascididemin (compound **3**) exhibits a very high MTD (> 160 mg/kg/day) but weak cytotoxic potency ($IC_{50} = 3200$ nM).

In a recent paper, Copp's group report various pyridine ring E analogues of ascididemin in an attempt to determine the pharmaceutical utility and the structure−activity requirements for the parent alkaloid.[13] All of the compounds were evaluated for selective cytotoxicity on a wide range of biological screenings. In vitro, some of these compounds exhibit cytotoxic levels similar to ascididemin for human solid tumor cell lines, while they failed to show any antitumor activity in in vivo xenograft assays.

In the present study, we are interested in ring D-modified ascididemins. The data that were obtained in vitro show that most of the compounds have cytotoxic properties, with some of them having levels of antitumor activity up to 100 times higher than the parent ascididemin (see Table 2). A large set of experiments are now under way in order to characterize the mechanism(s) of action of these new potential anticancer agents. All of the ring D-modified ascididemin analogues at least have similar cytotoxic effect or, more importantly, appear to be more cytotoxic than the reference compound ascididemin. In addition, when substituted on ring D, these ascididemin analogues exhibit a significantly greater MTD (160 mg/kg instead of 20 mg/kg for ascididemin and 40 mg/kg for 2-bromoleptoclinidone), which anticipates a better therapeutic index (antitumor efficacy/overall safety ratio) in in vivo models. Besides, it seems that both the R_1 and the R_3 positions on ring D could be modulated without losing the cytotoxic activity.

The Copp group, who investigated a series of ascididemin analogues, has shown that ascididemin can act to damage DNA oxidatively via a thiol-dependent conversion of oxygen into DNA-cleaving oxygen radicals.[16] These authors found a qualitative agreement between the compound reduction potential observed and the percentage of DNA cleavage. At the same time, a trend in the direction of a statistically significant correlation was observed between the reduction potential and the in vitro cytotoxicity in the case of the murine leukemia cell line P388. The same relation had been evidenced before, in the case of quinones derived from spectronigrin.[21] The results obtained from a study of 22 quinones indicated that there is a quantitative linear relationship between their reduction potentials and the rate at which they degrade DNA in vitro under identical experimental conditions. We also measured the redox potential of some of the ascididemin analogues that we prepared, but we failed to evidence any relationship between the redox potential and the cytotoxicity of these different compounds (data not shown).

In conclusion, we have succeeded in synthesizing ascididemin analogues, some of which have levels of antitumor cytotoxic activity equivalent to, or up to 100 times greater than, the natural parent product when directed against a panel of 12 human cancer cell lines (including solid tumor models of glioblastomas and

bladder, colon, breast, and lung cancers). Moreover, the 11 modified ring D analogues of ascididemin that we constructed exhibit a significantly better cytotoxic potency/systemic tolerability ratio, as indicated by a dramatic improvement of the MTD, increasing from 20 to 40 mg/kg (natural ascididemin/2-bromoleptoclinidone) to at least 160 mg/kg.

Experimental Section

Chemistry. Chemical Synthesis. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained with a JEOL 400 MHz spectrometer, with the chemical shifts in the remaining protons of the deuterated solvents serving as internal standards. IR spectra were obtained with a Perkin-Elmer (1600 series FTIR) spectrometer. Mass spectra (MS) were recorded on an automass Unicam spectrometer. Reagents were purchased from commercial sources and used as received. Chromatography was performed on silicagel (15−40 μm) by means of the solvent systems indicated below. The purity of the different ascididemin analogues was evaluated on two analytical chromatographic systems. System I consisted of a Luna phenylhexyl, 5 mm column (150 mm × 4.6 mm), H_2O/CH_3CN 65:35 at 1 mL/min flow rate, 250 nm, and system II consisted of a Zorbax-NH_2, 5 mm column (150 mm × 4.6 mm), isooctane/EtOH/MeOH 80:10:10 at 2 mL/min flow rate, 250 nm.

***N*-(2-Acetyl-4-dibenzylaminophenyl)acetamide.** A mixture of *N*-(2-acetyl-4-aminophenyl)acetamide[22] (1 g, 5.21 mmol), benzyl bromide (1.96 g, 11.46 mmol), and K_2CO_3 (1.58 g) in DMF (8 mL) was refluxed for 2 h. After DMF was removed over a vacuum, water (10 mL) was added and the reaction media was extracted with CH_2Cl_2 (3 × 20 mL). The organic layers were washed with brine, dried over $MgSO_4$, and concentrated to dryness. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 99:1) to give the dibenzyl derivative (1.68 g, 87%) as a yellow solid; mp 153 °C. ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 3H); 2.39 (s, 3H); 4.72 (s, 4H); 7.05 (dd, 1H, J = 9.6 and 3 Hz); 7.12 (d, 1H, J = 3 Hz); 7.34 (m, 10H); 8.51 (d, 1H, J = 9.6 Hz); 11.16 (s, 1H).

5-Benzylamino-2-aminoacetophenone. A mixture of *N*-(2-acetyl-4-dibenzylaminophenyl)acetamide (1.48 g, 3.98 mmol) in concentrated HCl (30 mL) was refluxed for 3 h. After it was cooled, the reaction media was pourred into ice-water (100 mL), neutralized with 5 N NaOH, and extracted with CH_2Cl_2 (4 × 100 mL). The organic layers were dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 99:1) to give the benzyl derivative as a yellow solid (0.63 g, 66%). ^{1}H NMR ($CDCl_3$): δ 2.45 (s, 3H); 4.27 (s, 2H); 4.75 (br. s, 1H); 5.77 (br. s, 2H); 6.55 (d, 1H, J = 8.4 Hz); 6.76 (dd, 1H, J = 8.4 and 2.4 Hz); 6.92 (d, 1H, J = 2.4 Hz); 7.22−7.38 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 28.28, 49.92, 114.60, 118.97, 123.25, 127.59, 127.90, 128.81, 138.81, 139.75, 143.59, 200.90.

Formation of Tricyclic Intermediates. General Method A. A solution of quinoline-5,8-dione in ethanol was dropped into a solution of cerium(III) chloride and aminoacetophenone in ethanol. The reaction media was stirred at room temperature overnight, hydrolyzed with 10% acetic acid, and extracted with $CHCl_3$. The organic layers were dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by flash chromatography to give the expected product.

6-(2-Acetyl-4-chloro-phenylamino)quinoline-5,8-dione (A14). Method A was employed using quinoline-5,8-dione (0.188 g, 1.18 mmol), cerium chloride (0.58 g, 2.36 mmol), 5-chloro-2-aminoacetophenone[23] (0.4 g, 3.14 mmol), ethanol (10 + 4 mL), and acetic acid (25 mL). Flash chromatography (CH_2Cl_2/MeOH 95:5) gave compound **A14** as a red solid (0.3 g, 78%); mp 210 °C. ^{1}H NMR ($CDCl_3$): δ 2. 65 (s, 3H); 6.84 (s, 1H); 7.52 (dd, 1H, J = 8.8 Hz); 7.57 (d, 1H, J = 8.8 Hz); 7.63 (dd, 1H, J = 8.0 and 4.4 Hz); 7.89 (d, 1H, J = 2.4 Hz); 8.46 (dd, 1H, J = 0.8 and 8.0 Hz); 9.02 (dd, 1H, J = 2.0 and 5.2 Hz); 11.18 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 28.5, 107.36, 121.86, 126.69, 126.85, 127.49, 128.39, 132.10, 134.06, 134.75, 138.36, 143.29, 148.32, 155.22, 181.28, 182.63, 200.39.

6-(2-Acetyl-4-methyl-phenylamino)quinoline-5,8-dione (A15). Method A was employed using quinoline-5,8-dione (0.215 g, 1.35 mmol), cerium chloride (0.67 g, 2.70 mmol), 5-methyl-2-aminoacetophenone[24] (0.42 g, 2.70 mmol), ethanol (12 + 5 mL), and acetic acid (30 mL). Flash chromatography (CH_2Cl_2/MeOH 95:5) gave compound **A15** as a red solid (0.41 g, 98%); mp 252 °C. ^{1}H NMR ($CDCl_3$): δ 2.42 (s, 3H); 2.77 (s, 3H); 6.86 (s, 1H); 7.38 (dd, 1H, J = 8 et 1.6 Hz); 7.52 (d, 1H, J = 8.0 Hz); 7.61 (dd, 1H, J = 5.2 and 7.6 Hz); 7.74 (d, 1H, J = 1.6 Hz); 8.46 (dd, 1H, J = 7.6 and 5.2 Hz); 9.02 (dd, 1H, J = 2 and 5.2 Hz); 11.18 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 20.91, 28.50, 106.40, 120.88, 126.63, 127.53, 132.69, 133.35, 134.66, 134.83, 137.19, 143.72, 148.58, 155.08, 181.57, 182.64, 201.53.

6-(2-Acetyl-4-methoxy-phenylamino)quinoline-5,8-dione (A16). Method A was employed using quinoline-5,8-dione (3.51 g, 22.08 mmol), cerium chloride (10.9 g, 44.03 mmol), 5-methoxy-2-aminoacetophenone[14] (7.29 g, 44.18 mmol), ethanol (200 + 90 mL), and acetic acid (500 mL). Flash chromatography (CH_2Cl_2/MeOH 95:5) gave compound **A16** as a red solid (4.25 g, 60%). ^{1}H NMR ($CDCl_3$): δ 2.65 (s, 3H); 3.87 (s, 3H); 6.76 (s, 1H); 7.12 (dd, 1H, J = 2.8 and 8.8 Hz); 7.42 (d, 1H, J = 2.8 Hz); 7.55 (d, 1H, J = 8.8 Hz); 7.61 (dd, 1H, J = 7.6 and 4.4 Hz); 8.45 (dd, 1H, J = 1.6 and 7.6 Hz); 9.01 (dd, 1H, J = 1.6 and 4.4 Hz); 10.80 (s, 1H).

6-(2-Acetyl-4-dimethylamino-phenylamino)quinoline-5,8-dione (A17). Method A was employed using quinoline-5,8-dione (0.36 g, 2.26 mmol), cerium chloride (1.1 g, 4.49 mmol), 5-(dimethylamino)-2-aminoacetophenone[25] (0.8 g, 4.49 mmol), ethanol (20 + 10 mL), and acetic acid (50 mL). Flash chromatography (CH_2Cl_2/MeOH 95:5) gave compound **A17** as a red solid (0.65 g, 84%). ^{1}H NMR ($CDCl_3$): δ 2.85 (s, 3H); 3.12 (s, 6H); 6.72 (s, 1H); 6.90 (dd, 1H, J = 2.8 and 9.2 Hz); 7.15 (d, 1H, J = 2.8 Hz); 7.49 (d, 1H, J = 9.2 Hz); 7.58 (dd, 1H, J = 8.0 and 4.4 Hz); 8.43 (dd, 1H, J = 1.6 and 8.0 Hz); 9.00 (dd, 1H, J = 1.6 and 4.4 Hz); 10.69 (s, 1H).

6-(2-Acetyl-4-benzylamino-phenylamino)quinoline-5,8-dione (A18). Method A was employed using quinoline-5,8-dione (0.25 g, 1.57 mmol), cerium chloride (0.77 g, 3.14 mmol), 5-benzylamino-2-aminoacetophenone (0.60 g, 3.14 mmol), ethanol (15 + 7 mL), and acetic acid (35 mL). Flash chromatography (CH_2Cl_2/MeOH 95:5) gave compound **A18** as a red solid (0.56 g, 91%), which decomposed before melting. ^{1}H NMR ($CDCl_3$): δ 2.54 (s, 3H); 4.38 (s, 2H); 6.70 (s, 1H); 6.83 (dd, 1H, J = 9.6 and 3.2 Hz); 7.08 (d, 1H, J = 3.2 Hz); 7.30−7.37 (m, 5H); 7.43 (d, 1H, J = 9.6 Hz); 7.58 (dd, 1H, J = 7.6 and 4.8 Hz); 8.43 (dd, 1H, J = 7.6 and 2.0 Hz); 9.03 (dd, 1H, J = 2.0 and 4.8 Hz); 10.67 (s, 1H).

Formation of Tetracyclic Intermediates. General Method B. A solution of concentrated sulfuric acid in acetic acid was dropped into a solution of tricyclic compound obtained according to general procedure A. The reaction media was refluxed for 30 min, and after it was cooled, it was poured into ice. The mixture was neutralized with NH_4OH and extracted four times with CH_2Cl_2. The organic layers were dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by flash chromatography to give the expected tetracyclic compound.

9-Chloro-11-methyl-1,6-diaza-naphthacene-5,12-dione (B14). Method B was employed using tricyclic compound **A14** (0.289 g, 0.88 mmol), H_2SO_4 cc (1.3 mL), and acetic acid (8 + 6.5 mL). Flash chromatography (CH_2Cl_2/MeOH 95:5) gave the tetracyclic compound **B14** as a brown solid (0.26 g, 95%); mp > 260 °C. ^{1}H NMR ($CDCl_3$): δ 3.25 (s, 3H); 7.76 (dd, 1H, J = 8.0 and 4.8 Hz); 7.85 (dd, 1H, J = 8.8 and 2.0 Hz); 8.33 (d, 1H, J = 2.0 Hz); 8.38 (d, 1H, J = 8.8); 8.71 (dd, 1H, J = 1.6 and 8.0 Hz); 9.15 (dd, 1H, J = 1.6 and 4.8 Hz). ^{13}C NMR ($CDCl_3$): δ 16.75, 124.67, 126.10, 128.05, 130.10, 130.65, 133.79, 133.84, 135.86, 136.50, 147.11, 147.68, 150.15, 151.76, 155.84, 181.59, 183.26.

9,11-Dimethyl-1,6-diaza-naphthacene-5,12-dione (B15). Method B was employed using tricyclic compound **A15** (0.4 g, 1.3 mmol), H_2SO_4 cc (1.9 mL), and acetic acid (12 + 9.6 mL). Flash chromatography (CH_2Cl_2/MeOH 95:5) gave the tetracyclic compound **B15** as a dark-yellow solid (0.33 g, 86%); mp > 260 °C. ^{1}H NMR ($CDCl_3$): δ 2.64 (s, 3H); 3.29 (s, 3H); 7.74 (dd, 1H, J = 7.6 and 4.8 Hz); 7.75 (dd, 1H, J = 8.4 and 1.6 Hz); 8.12 (dd, J = 1.6 Hz); 8.33 (d, 1H, J = 8.4); 8.71 (dd, 1H,

J = 2.0 and 7.6 Hz); 9.13 (dd, 1H, J = 2.0 and 4.8 Hz). ^{13}C NMR ($CDCl_3$): δ 16.63, 22.37, 124.48, 125.68, 127.85, 129.91, 130.10, 132.08, 135.17, 135.76, 140.58, 146.91, 147.32, 150.31, 151.69, 155.60, 181.95, 183.61.

9-Methoxy-11-methyl-1,6-diaza-naphthacene-5,12-dione (B16). Method B was employed using tricyclic compound **A16** (4.25 g, 13.18 mmol), H_2SO_4 cc (20 mL), and acetic acid (110 + 100 mL). Flash chromatography (CH_2Cl_2/MeOH 100:3) gave the tetracyclic compound **B16** (which was washed with diethyl ether) as a yellow solid (2.9 g, 72%); mp > 260 °C. ^{1}H NMR ($CDCl_3$): δ 3.25 (s, 3H); 4.02 (s, 3H); 7.49 (d, 1H, J = 3.3 Hz); 7.56 (dd, 1H, J = 3.3 and 9.3 Hz); 7.74 (dd, 1H, J = 8.3 and 4.3 Hz); 8.34 (d, 1H, J = 9.3 Hz); 8.71 (dd, 1H, J = 2.5 and 8.3 Hz); 9.12 (dd, 1H, J = 2.5 and 4.3 Hz). ^{13}C NMR ($CDCl_3$): δ 16.65, 55.81, 103.02, 125.73, 125.83, 127.83, 130.03, 131.47, 133.90, 135.69, 144.87, 145.46, 149.89, 150.19, 155.47, 160.54, 181.74, 183.77.

9-Dimethylamino-11-methyl-1,6-diaza-naphthacene-5,-12-dione (B17). Method B was employed using tricyclic compound **A17** (0.76 g, 2.27 mmol), H_2SO_4 cc (3.5 mL), and acetic acid (20 + 18 mL). Tetracyclic compound **B17** was washed with diethylic ether after workup without further purification; it was brown solid (0.76 g, 93%); mp > 260 °C. ^{1}H NMR ($CDCl_3$): δ 3.17 (s, 3H); 3.21 (s, 6H); 7.04 (d, 1H, J = 3.2 Hz); 7.51 (dd, 1H, J = 3.2 and 9.2 Hz); 7.71 (dd, 1H, J = 8 and 4.4 Hz); 8.26 (d, 1H, J = 9.2 Hz); 8.7 (dd, 1H, J = 1.6 and 8.0 Hz); 9.09 (dd, 1H, J = 1.6 and 4.4 Hz). ^{13}C NMR ($CDCl_3$): δ 16.40, 40.47 (2C), 101.39, 121.73, 126.11, 127.66, 130.32, 131.95, 133.50, 135.60, 142.37, 147.84, 150.40, 150.52, 155.10, 181.84, 184.38.

9-Benzylamino-11-methyl-1,6-diaza-naphthacene-5,12-dione (B18). Method B was employed using tricyclic compound **A18** (4.0 g, 10 mmol), H_2SO_4 cc (15.1 mL), and acetic acid (92 + 75 mL). The tetracyclic compound **B18** was just washed with diethyl ether after workup without further purification; black solid (3.58 g, 98%); mp > 260 °C. ^{1}H NMR ($CDCl_3$): δ 3.09 (s, 3H); 4.52 (d, 2H, J = 5.4 Hz); 4.86 (t, 1H, J = 5.4 Hz); 7.06 (d, 1H, J = 2.8 Hz); 7.29 (dd, 1H, J = 9.2 and 2.8 Hz); 7.3−7.43 (m, 5H); 7,71 (dd, 1H, J = 4.8 and 8 Hz); 8.20 (d, 1H, J = 9.8 Hz); 8.69 (dd,1H, J = 1.6 and 8 Hz); 9.09 (dd, 1H, J = 1.6 and 4.8 Hz).

Formation of Pentacycles. General Method C. A solution of tetracyclic intermediate obtained according to general method B and DMF-DEA in DMF was refluxed for 1 h. After it was concentrated to dryness, NH_4Cl and ethanol were added and the mixture was refluxed for an additional 30 min. The solvent was removed over vacuum, water was added, and the mixture was extracted four times with CH_2Cl_2. The organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified to give the expected pentacyclic derivative.

7-Nitro-9*H*-quino[4,3,2-*de*][1,10]phenanthrolin-9-one (6). Ascididemin (2 g, 7.06 mmol) was dropped at 0 °C into a solution of sulfuric acid (45 mL) and nitric acid (45 mL). The reaction mixture was warmed at 130 °C for 2 h, and after it was cooled, it was poured into ice (400 g). The precipitate was recovered by filtration, and a mixture $CH_2Cl_2/NH_4OH/H_2O$ (600:1:300) was added. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The organic extracts were dried on $MgSO_4$ and concentrated over a vacuum to give the pentacyclic compound **6** as a yellow solid (1.62 g, 70%); mp 224 °C. ^{1}H NMR ($CDCl_3$): δ 7.69 (dd, 1H, J = 4.4 and 8 Hz); 8.04 (dd, 1H, J = 8.0 and 8.0 Hz); 8.28 (d, 1H, J = 8 Hz); 8.56 (d, 1H, J = 5.2 Hz); 8.75 (dd, 1H, J = 2.0 and 8.0 Hz); 8.89 (dd, 1H, J = 1.2 and 8 Hz); 9.18 (dd, 1H, J = 4.4 and 2.0 Hz); 9.37 (d, 1H, J = 5.6 Hz). ^{13}C NMR ($CDCl_3$): δ 79.20, 117.61, 118.39, 124.21, 124.89, 125.98, 127.54, 129.04, 130.14, 135.62, 136.63, 148.17, 149.76, 149.94, 150.12, 151.66, 154.88, 180.56. IR ($CHCl_3$): 1689 cm^{-1}. MS (m/z): 328 (18); 327 (100); 299 (22); 297 (9); 269 (10); 253 (24); 242 (11); 241 (33). t_R is 8.51 min (97% purity), using system I, and t_R is 11.99 min (100% purity), using system II.

7-Amino-9*H*-quino[4,3,2-*de*][1,10]phenanthrolin-9-one (8). A suspension of nitro-derivative **6** (0.4 g, 1.22 mmol) and iron (0.37 g, 6.59 mmol) in AcOH/H_2O (10 mL:10 mL) was refluxed for 1 h. Ethylenediaminetetraacetic acid (EDTA, 1.94 g, 6.59 mmol) was added, and the reaction media was made alkaline by the addition of concentrated NaOH. The mixture was extracted with CH_2Cl_2, and the organic layer was dried over $MgSO_4$ and concentrated to dryness to give the expected amino compound as a blue solid (0.32 g, 88%); mp > 260 °C. ^{1}H NMR ($CDCl_3$): δ 5.68 (s, 2H); 7.16 (d, 1H, J = 7.8 Hz); 7.66 (dd, 1H, J = 7.6 and 4.8 Hz); 7.69 (dd, 1H, J = 7.8 and 7.8 Hz); 7.91 (d, 1H, J = 7.8 Hz); 8.46 (d, 1H, J = 5.2 Hz); 8.77 (dd, 1H, J = 1.6 and 7.6 Hz); 9.17 (dd, 1H, J = 1.6 and 4.8 Hz); 9.21 (d, 1H, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$): δ 109.42, 112.71, 117.70, 118.43, 124.29, 125.64, 129.12, 132.63, 132.81, 135.53, 137.27, 141.68, 148.68, 148.89, 149.03, 151.96, 154.68, 180.71. IR ($CHCl_3$) 3510, 3400, 1676 cm^{-1}. MS (m/z): 298 (35); 297 (100); 269 (11); 268 (8). t_R was 3.47 min (99% purity) using system I and 4.99 min (97% purity) using system II.

5-Bromo-9*H*-quino[4,3,2-*de*][1,10]phenantrolin-9-one (9). A solution of bromine (0.2 mL, 3.88 mmol) in acetic acid (5 mL) was dropped into a solution of ascididemin (0.5 g, 1.77 mmol) in acetic acid (20 mL). The reaction was refluxed for 24 h. After it was cooled, the mixture was neutralized with a $NaHCO_3$ saturated solution and extracted four times by CH_2Cl_2. The organic extracts were dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 96:4) to give the bromo-derivative as a yellow solid (0.548 g, 86%); mp 208 °C. ^{1}H NMR ($CDCl_3$): δ 7.68 (dd, 1H, J = 4.4 and 8 Hz); 8.09 (dd, 1H, J = 8.8 and 2.0 Hz); 8.48 (d, 1H, J = 8.8 Hz); 8.49 (d, 1H, J = 6.0 Hz); 8.79 (dd, 1H, J = 2.0 and 8.0 Hz); 8.82 (d, 1H, J = 2.0 Hz); 9.18 (dd, 1H, J = 2.0 and 4.4 Hz); 9.30 (d, 1H, J = 6.0 Hz). ^{13}C NMR ($CDCl_3$): δ 116.76, 117.04, 118.26, 124.76, 125.81, 125.93, 129.05, 134.52, 135.43, 136.72, 137.01, 144.41, 146.24, 149.93, 150.12, 152.27, 155.67, 181.69. IR ($CHCl_3$) 1684 cm^{-1}. MS (m/z): 363 (99); 362 (83); 361 (100); 360 (27); 255 (9); 254 (51). t_R was 10.73 min (97% purity) using system I and 3.52 min (99% purity) using system II.

5-Amino-9*H*-quino[4,3,2-*de*][1,10]phenanthrolin-9-one (10). A solution of bromoascididemin **9** (9.0 g, 24.9 mmol) and NaN_3 (9.0 g, 120 mmol) in DMF (800 mL) was refluxed for 9 h. DMF was removed over vacuum, and 1 N KOH and $CHCl_3$/MeOH 95:5 (500 mL) were added. The mixture was filtered to recover the precipitate, and the organic layer was extracted by $CHCl_3$/MeOH 95:5 (5 × 100 mL). The organic layers were dried over $MgSO_4$ and concentrated to give a solid, which was added to the previous precipitate. These solids were washed by $CHCl_3$ and recrystallized in MeOH to give the amino compound as a black solid (6.5 g, 88%); mp > 260 °C. ^{1}H NMR ($CDCl_3$): δ 7.43 (dd, 1H, J = 8.8 and 2.4 Hz); 7.74 (dd, 1H, J = 4.8 and 8.0 Hz); 7.81 (d, 1H, J = 2.4 Hz); 8.48 (d, 1H, J = 6.0 Hz); 8.50 (d, 1H, J = 8.8 Hz); 8.90 (dd, 1H, J = 2.0 and 8.0 Hz); 9.25 (dd, 1H, J = 2.0 and 4.8 Hz); 9.29 (d, 1H, J = 6.0 Hz).^{13}C NMR (DMSO): δ 102.26, 117.13, 118.54, 121.62, 123.20, 125.34, 126.11, 129.18, 133.80, 134.83, 135.47, 138.42, 147.65, 148.29, 151.63, 152.39, 154.32, 180.35. IR (KBr) 3312, 3200, 1692 cm^{-1}. MS (m/z): 298 (32); 297 (100); 269 (4); 268 (1). t_R was 2.56 min (100% purity) using system I and 10.6 min (97% purity) using system II.

5-(2-Chloroethylamino)-9*H*-quino[4,3,2-*de*][1,10]phenanthrolin-9-one (11) and 5-Bis(2-chloroethylamino)-9*H*-quino[4,3,2-*de*][1,10]phenanthrolin-9-one (12). $NaBH_3CN$ (0.63 g, 10 mmol) was added portionwise at 0 °C into a solution of aminoascididemin **10** (1 g, 3.95 mmol) and chloroacetaldehyde (50% in water, 2.6 mL, 16.8 mmol) in acetic acid (30 mL). The reaction was maintained at 0 °C for 5 min and at room temperature for a further 30 min. The mixture was made alkaline by the addition of an $NaHCO_3$-saturated solution and extracted by $CHCl_3$/MeOH 95:5. The organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography ($CHCl_3$ and then $CHCl_3$/MeOH 99:1) to give compounds **11** and **12**.

Compound 11. Purple solid (0.22 g, 18%); mp 196 °C. ^{1}H NMR ($CDCl_3$): δ 3.81 (t, 2H, J = 5.5 Hz); 3.88 (t, 2H, J = 5.5 Hz); 5.01 (br.s, 1H); 7.34 (dd, 1H, J = 8.8 and 2.5 Hz); 7.60 (d, 1H, J = 2.5 Hz); 7.65 (dd, 1H, J = 7.5 and 4.4 Hz); 8.41 (d, 1H, J = 5.8 Hz); 8.43 (d, 1H, J = 8.8 Hz); 8.82 (dd, 1H, J = 7.5 and 1.5 Hz); 9.15 (dd, 1H, J = 4.4 and 1.5 Hz); 9.21 (dd, 1H, J = 5.8 Hz). ^{13}C NMR ($CDCl_3$): δ 42.83, 45.01, 100.76, 116.81, 118.78, 120.85, 125.38, 126.35, 129.35, 135.04, 136.04, 136.43, 140.22, 141.56, 148. IR ($CHCl_3$): 3427, 1672, 1620

cm^{-1}. t_R was 5.22 min (98% purity) using system I and 5.83 min (99% purity) using system II.

Compound 12. Pink solid (0.14 g,10%); mp 220 °C. ^1H NMR ($CDCl_3$): δ 3.83 (t, 4H, J = 7.0 Hz); 4.04 (t, 4H, J = 7.0 Hz); 7.47 (dd, 1H, J = 9.5 and 2.9 Hz); 7.66 (dd, 1H, J = 8.0 and 4.4 Hz); 7.70 (d, 1H, J = 2.9 Hz); 8.42 (d, 1H, J = 5.6 Hz); 8.50 (d, 1H, J = 9.5 Hz); 8.81 (dd, 1H, J = 8.0 and 1.8 Hz); 9.16 (dd, 1H, J = 4.4 and 1.8 Hz); 9.23 (d, 1H, J = 5.6 Hz). ^{13}C NMR ($CDCl_3$): δ 40.16, 53.60, 101.70, 116.60, 118.37, 118.68, 125.39, 125.91, 129.25, 135.13, 136.12, 136.38, 139.42, 141.93, 148.24, 148.73, 149.34, 152.22, 155.08, 181.43. IR (KBr): 1666; 1650 cm^{-1}. t_R was 3.39 min (97% purity) using system I and 4.82 min (100% purity) using system II.

5-(Dimethylamino-2-ethylamino)-9*H*-quino[4,3,2-*de*]-[1,10]phenanthrolin-9-one (13). Trifluoroacetic acid (25 mL, 166 mmol) was added dropwise at 0 °C into a mixture of compound **10** (2.56 g, 8.59 mmol) and dimethylaminoacetaldehyde diethyl acetal (7.7 mL, 43.3 mmol). The reaction was stirred for 5 min, and sodium cyanoborohydride (8.2 g, 130 mmol) was added portionwise. The reaction was warmed at 95 °C for 18 h. An $NaHCO_3$-saturated solution (600 mL) was added, and the mixture was extracted by $CHCl_3$/MeOH 95:5 (3 × 800 mL). The organic layers were washed with water and dried over $MgSO_4$. The solvent was removed over a vacuum, and the crude product was purified by filtration on alumina ($CHCl_3$, $CHCl_3$/MeOH 95:5) to give compound **13** as a black solid (1.15 g, 36%), which decomposes before melting. ^1H NMR ($CDCl_3$): δ 2.37 (s, 6H); 2.62 (t, 2H, J = 7.32 Hz); 3.70 (t, 2H, J = 7.32 Hz); 7.39 (dd, 1H, J = 9.2 and 3.0 Hz); 7.62 (dd, 1H, J = 8.0 and 4.5 Hz); 7.66 (d, 1H, J = 3.0 Hz); 8.35 (d, 1H, J = 9.2 Hz); 8.38 (d, 1H, J = 5.7 Hz); 8.79 (dd, 1H, J = 8.0 and 1.8 Hz); 9.12 (dd, 1H, J = 4.5 and 1.8 Hz); 9.15 (d, 1H, J = 5.7 Hz). ^{13}C NMR ($CDCl_3$): δ 45.97, 50.31, 56.40, 101.05, 116.81, 118.48, 118.89, 125.22, 126.30, 129.35, 134.87, 135.97, 136.32, 138.91, 140.55, 148.25, 148.98, 149.69, 152.23, 154.82, 181.37. IR ($CHCl_3$): 1663 cm^{-1}. MS: m/z 369 (100); 354 (15); 236 (37). t_R was 10.37 min (98% purity) using system I and 6.86 min (97% purity) using system II.

5-Chloro-9*H*-quino[4,3,2-*de*][1,10]phenantrolin-9-one (14). Method C was employed using tetracyclic compound **B14** (0.25 g, 0.81 mmol) and dimethylformamide diethylacetal (1.5 mL, 8.75 mmol) in DMF (4.5 mL), NH_4Cl (2.95 g, 55 mmol), and ethanol (50 mL). The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to give compound **14** as a yellow solid (60 mg, 23%); mp 200 °C. ^1H NMR ($CDCl_3$): δ 7.68 (dd, 1H, J = 8.4 and 4.8 Hz); 7.94 (dd, 1H, J = 8.8 and 2.0 Hz); 8.46 (d, 1H, J = 5.6 Hz); 8.55 (d, 1H, J = 8.8 Hz); 8.63 (d, 1H, J = 2.0 Hz); 8.79 (dd, 1H, J = 2.0 and 8.4 Hz); 9.18 (dd, 1H, J = 4.8 and 2.0 Hz); 9.30 (d, 1H, J = 5.6 Hz).^{13}C NMR ($CDCl_3$): δ 117.07, 118.46, 122.98, 124.82, 126.12, 129.34, 133.02, 134.81, 137.00, 137.42, 137.79, 144.45, 146.35, 150.24, 150.45, 152.55, 156.02, 181.9. IR ($CHCl_3$): 1684, 1602 cm^{-1}. MS (m/z): 319 (43); 318 (15); 317 (100); 291 (14,5); 290 (18); 289 (100). t_R was 8.30 min (96% purity) using system I and 3.58 min (97% purity) using system II.

5-Methyl-9*H*-quino[4,3,2-*de*][1,10]phenanthrolin-9-one (15). Method C was employed using tetracyclic compound **B15** (1.0 g, 3.47 mmol) and dimethylformamide diethylacetal (1.8 mL, 10.41 mmol) in DMF (7 mL), NH_4Cl (2.77 g, 52 mmol), and ethanol (50 mL). The recrystallization of the crude product gave compound **15** as a yellow solid (0.7 g, 67%); mp 200 °C. ^1H NMR ($CDCl_3$): δ 2.69 (s, 3H); 7.65 (dd, 1H, J = 8.0 and 4.8 Hz); 7.81 (dd, 1H, J = 8.0 and 1.2 Hz); 8.44 (d, 1H, J = 1.2 Hz); 8.49 (d, 1H, J = 8.0 Hz); 8.50 (d, 1H, J = 5.6 Hz); 8.78 (dd, 1H, J = 2.0 and 8.0 Hz); 9.15 (dd, 1H, J = 4.8 and 2.0 Hz); 9.24 (d, 1H, J = 5.6 Hz). ^{13}C NMR ($CDCl_3$): δ 22.06, 116.54, 117.87, 122.15, 123.12, 125.24, 128.74, 132.58, 133.47, 136.25, 137.19, 141.63, 143.88, 144.79, 149.16, 149.31, 152.09, 155.15, 181.53. IR ($CHCl_3$): 1681, 1622 cm^{-1}. MS (m/z): 297 (18); 296 (34); 268 (25); 149 (50). t_R was 5.44 min (99% purity) using system I and 3.88 min (100% purity) using system II.

5-Methoxy-9*H*-quino[4,3,2-*de*][1,10]phenanthrolin-9-one (16). Method C was employed using tetracyclic compound **B16** (2.0 g, 6.57 mmol) and dimethylformamide diethylacetal (4 mL, 23.34 mmol) in DMF (14 mL), NH_4Cl (8 g, 149.5 mmol), and ethanol (130 mL). The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 100: 5) to give compound **16** as a green solid (170 mg, 66%); mp > 260 °C. ^1H NMR ($CDCl_3$): δ 4.10 (s, 3H); 7.62 (dd, 1H, J = 9.2 Hz, J = 2.4 Hz); 7.66 (dd, 1H, J = 4.4 and 8.0 Hz); 7.96 (d, 1H, J = 2.4 Hz); 8.48 (d, 1H, J = 2.4 Hz); 8.54 (d, 1H, J = 9.2 Hz); 8.80 (dd, 1H, J = 2.4 and 8.0 Hz); 9.16 (dd, 1H, J = 4.4 and 2.4 Hz); 9.25 (d, 1H, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$): δ 30.93, 116.86, 118.41, 122.44, 125.56, 129.25, 134.96, 136.55, 137.13, 141.52, 143.67, 149.11, 149.77, 152.37, 155.38, 161.71, 181.93, 207.00. IR ($CHCl_3$): 1677, 1616 cm^{-1}. MS (m/z): 313 (26); 312 (100); 285 (2); 284 (15); 269 (15); 242 (33). t_R was 4.93 min (99% purity) using system I and 5.09 min (100% purity) using system II.

5-(Dimethylamino)-9*H*-quino[4,3,2-*de*][1,10]phenanthrolin-9-one (17). Method C was employed using tetracyclic compound **B17** (0.25 g, 0.79 mmol) and dimethylformamide diethylacetal (1.5 mL, 8.75 mmol) in DMF (5 mL), NH_4Cl (1 g, 18.7 mmol), and ethanol (16 mL). The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 100: 5) to give compound **17** as a purple solid (170 mg, 66%); mp > 260 °C. ^1H NMR ($CDCl_3$): δ 3.25 (s, 6H); 7.45 (dd, 1H, J = 9.2 and 3.0 Hz); 7.57 (d, 1H, J = 3.0 Hz); 7.63 (dd, 1H, J = 4.4 and 8.0 Hz); 8.41 (d, 1H, J = 9.2 Hz); 8.43 (d, 1H, J = 5.6 Hz); 8.81 (dd, 1H, J = 2.0 and 7.6 Hz); 9.13 (dd, 1H, J = 4.4 and 2.0 Hz); 9.17 (d, 1H, J = 5.6 Hz). ^{13}C NMR ($CDCl_3$): δ 40.45, 100.84, 116.81, 118.69, 118.99, 125.19, 126.10, 129.46, 134.62, 136.03, 136.30, 139.00, 140.69, 148.16, 149.15, 151.53, 152.47, 154.83, 181.65. IR ($CHCl_3$): 1666, 1615 cm^{-1}. MS (m/z): 326 (35); 325 (100); 324 (100); 254 (16); 253 (14). t_R was 4.38 min (98% purity) using system I and 5.4 min (98% purity) using system II.

5-(Benzylamino)-9*H*-quino[4,3,2-*de*][1,10]phenanthrolin-9-one (18). Method C was employed using tetracyclic compound **B18** (3.58 g, 9.45 mmol), dimethylformamide diethylacetal (5.7 mL, 33.26 mmol) in DMF (19 mL), NH_4Cl (2.95 g, 55 mmol), and ethanol (50 mL). The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 96:4) to give compound **18** as a pink-purple solid (2 g, 55%); mp 219 ° C. ^1H NMR ($CDCl_3$): δ 4.61 (d, 2H); 5.10 (t, 1H); 7.31 (dd, 1H, J = 8.8 and 2.4 Hz); 7.452−7.327 (m, 5H); 7.55 (d, 1H, J = 2,4 Hz); 7.63 (dd, 1H, J = 4.4 and 8.4 Hz); 8.29 (d, 1H, J = 5.2 Hz); 8.36 (d, 1H, J = 8.8 Hz); 8.79 (dd, 1H, J = 1.2 and 8.4 Hz); 9.13 (dd, 1H, J = 4.4 and 1.2 Hz); 9.14 (d, 1H, J = 5.2 Hz). IR ($CHCl_3$): 3428, 1668, 1620 cm^{-1}. MS (m/z): 388 (7); 387 (100); 386 (85); 385 (25); 369 (99); 368(44). t_R was 11.03 min (97% purity) using system I and 5.01 min (99% purity) using system II.

4-Bromo-5-amino-9-*H*-quino[4,3,2-*de*][1,10]phenanthrolin-9-one (19). Bromine (35 mL, 0.67 mmol) was added into a suspension of compound **10** (0.2 g, 0.67 mmol) in acetic acid (8 mL). The reaction was warmed at 50 °C for 6 h. After it was concentrated, the mixture was made alkaline by the addition of 5 N NaOH (20 mL) and extracted by $CHCl_3$/MeOH 95:5 (4 × 100 mL). The organic layers were dried over $MgSO_4$ and concentrated to give compound **19,** which was recrystallized in $CHCl_3$/pentane 20 mL:15 mL (152 mg, 61%); mp > 260 °C. ^1H NMR (DMSO-d_6): δ 7.07 (br. s, 2H); 7.61 (d, 1H, J = 8.8 Hz); 7.77 (dd, 1H, J = 7.7 and 4.0 Hz); 8.18 (d, 1H, J = 8.8 Hz); 8.61 (d, 1H, J = 7.7 Hz); 9.10 (d, 1H, J = 4.0 Hz); 9.14 (d, 1H, J = 5.9 Hz); 9.91 (d, 1H, J = 5.9 Hz). IR ($CHCl_3$): 3501; 3400; 1673 cm^{-1}. MS: m/z 378 (42); 377 (100); 376 (48); 375 (27). t_R was 5.66 min (97% purity) using system I and 7.03 min (99% purity) using system II.

Pharmacology. In Vitro Characterization of the Drug-Induced Effects on Human Cancer Cell Line Growth. Twelve human tumor cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). These included three glioblastomas (SW1088, U-373 MG, and U-87 MG), two colon (HCT-15 and LoVo), two nonsmall-cell-lung (A549 and A-427), two bladder (J82 and T24), one prostate (PC-3), and two breast (T-47D and MCF7) cancer models. The ATCC numbers of these cell lines are HTB 12 (SW1088), HTB 14 (U-87 MG), HTB 17 (U-373 MG), CCL225 (HCT-15), CCL229 (LoVo), CCL 185 (A549), HBT 53 (A-427), HTB1 (J82), HTB4 (T24), HTB133 (T-47D), HTB22 (MCF7), and CRL1435 (PC-3). The cells were cultured at 37 °C in sealed (airtight) Falcon plastic dishes (Nunc, Gibco, Belgium) containing Eagle's minimal essential medium (MEM, Gibco)

supplemented with 5% fetal calf serum (FCS). All of the media were supplemented with a mixture of 0.6 mg/mL glutamine (Gibco), 200 IU/mL penicillin (Gibco), 200 IU/mL streptomycin (Gibco), and 0.1 mg/mL gentamycin (Gibco). The FCS was heat-inactivated for 1 h at 56 °C.

The 12 cell lines were incubated for 24 h in 96 microwell plates (at a concentration of 40 000 cells/mL culture medium) to ensure adequate plating prior to the determination of the cell growth. This process was carried out by means of the colorimetric MTT assay, as detailed previously.[26,27] This assessment of cell population growth is based on the capability of living cells to reduce the yellow product MTT (Sigma, St. Louis, MO) to a blue product, formazan, by a reduction reaction occurring in the mitochondria. The number of living cells is directly proportional to the intensity of the blue, which is quantitatively measured by spectrophotometry on a DIAS microplate reader (Dynatech Laboratories, Guyancourt, France) at a 570 nm wavelength (with a reference of 630 nm). Each experiment was carried out in sextuplicate. We validated the MTT-related data using two alternative techniques, namely, direct cell counting and the genomic incorporation of tritiated thymidine (data not shown). Six concentrations ranging from 10^{-5} to 10^{-9} M were assayed for each of the 17 compounds under study (see Table 2).

In Vivo Determination of Drug-Induced Toxicity. Drug-induced toxicity can be monitored in vivo by determining the MTD. This MTD determination is carried out by defining the maximum dose of the drug that can be administered acutely (i.e., in one intraperitoneal single dose) to healthy animals (B6D2F1 mice, Iffa Credo), i.e., not grafted with tumors. The survival and weight of the animals are recorded for up to 28 days postinjection. Six different doses of each drug (5, 10, 20, 40, 80, and 160 mg/kg) are used for the MTD index determination, with each experimental group being composed of three mice for this purpose.

Statistical Analysis. The statistical comparisons of the data were carried out by means of the Fisher *F* (one-way variance analysis for more than two groups) or the Student *t* (for two groups) tests after a check of the equality of variance by means of the Levene test and of the normal distribution fitting of the data by means of the c^2 test of goodness-of-fit. When these parametric conditions were not satisfied, the nonparametric Kruskall−Wallis (for more than two groups) or the Mann−Whitney (for two groups) tests were carried out. All of the statistical analyses were carried out using Statistica (Statsoft, Tulsa, OK).

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