Synthesis and in Vitro Antitumor Activity of Phenanthrolin-7-one Derivatives, Analogues of the Marine Pyridoacridine Alkaloids Ascididemin and Meridine: Structure−Activity Relationship

Evelyne Delfourne,*,† Robert Kiss,‡ Laurent Le Corre,† Frederic Dujols,† Jean Bastide,† Françoise Collignon,§ Brigitte Lesur,§ Armand Frydman,§ and Francis Darro§

Centre de Phytopharmacie, UMR−CNRS 5054, Université de Perpignan, 52 Avenue de Villeneuve, 66860 Perpignan Cedex, France, Laboratoire d'Histopathologie, Faculté de Médecine, Université Libre de Bruxelles, 808 Route de Lennik, 1070 Bruxelles, Belgique, and Laboratoire Louis Lafon, Centre de Recherches, 19 avenue du Pr Cadiot, B.P. 22, 94701 Maisons-Alfort Cedex, France

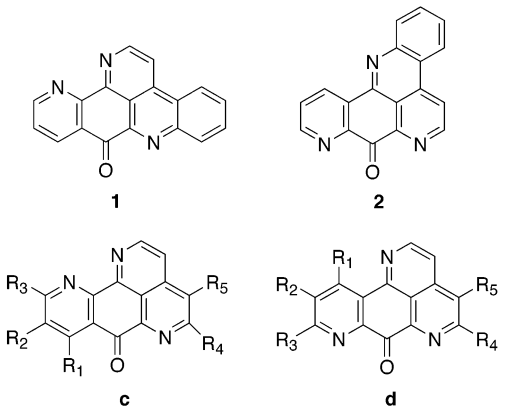
Received April 11, 2003

A series of substituted pyrido[4,3,2-*de*][1,7] or [1,10]-phenanthrolin-7-ones, analogues of the marine pyridoacridines meridine and ascididemin, have been synthesized on the basis of Diels−Alder reactions involving different quinoline-5,8-diones and *N*,*N*-aldehyde-dimethylhydrazones. All the compounds were evaluated for in vitro cytotoxic activity against 12 distinct human cancer cell lines. They all exhibit cytotoxic activity with IC_{50} values at least of micromolar order.

Introduction

Since the discovery of amphimedine in 1983[1] until recent isolation and characterization of kuanoniamines E and F in 2002,[2] more than 50 pyridoacridine alkaloids have been isolated from sponges and ascidians. A considerable amount of attention has been focused on this class of compounds due to their potentially valuable biological activity.[3]

Ascididemin **1** and meridine **2** are two members of the pyridoacridine family differing by the attachment of the benzene ring, the position of a nitrogen atom, and a hydroxy substituent in meridine. In a previous report,[4a] we have shown that this substituent was not necessary for biological activity, both the unsubstituted analogue of meridine and ascididemin presenting important cytotoxicity on different cell lines. So far, despite the results of structure−activity study reported by Lindsay et al.,[5] the minimal structural requirement for biological activity of these compounds remains unknown.



As part of our work on natural pyridoacridines as a source of new and useful anticancer drugs,[4] we have investigated the tetracyclic pyridophenanthrolin-7-one compounds **c** and **d**, of which the sole structures previously reported were the unsubstituted ones, by Matsumoto et al.[6] These skeletons constitute the common moiety of the marine pyridoacridine alkaloids **1** and **2**.

We describe herein the synthesis of these compounds and their in vitro antitumor activity and discuss the influence of the different substituents on this activity.

Chemistry

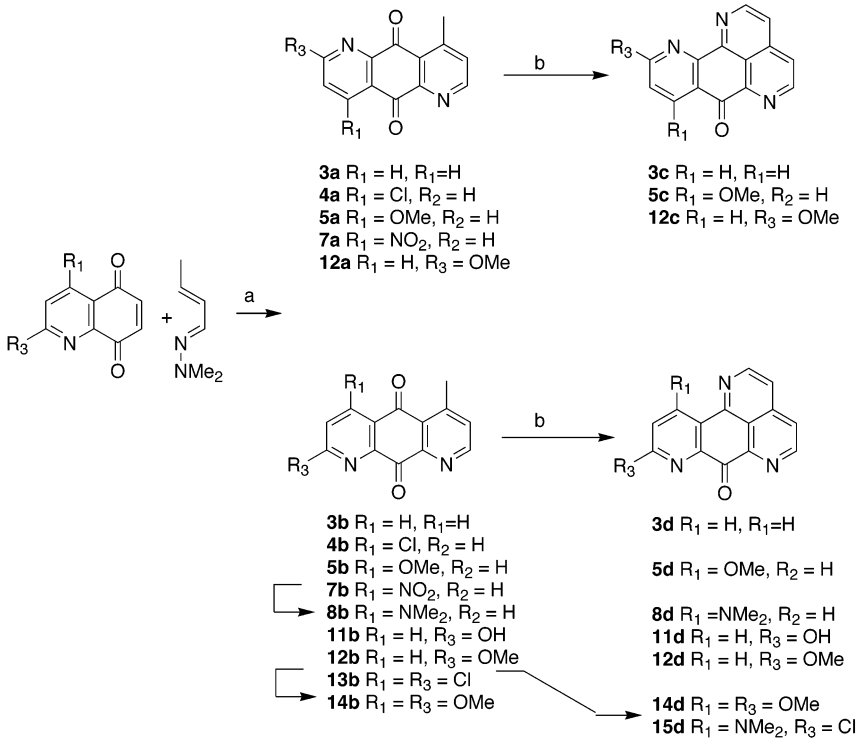
In a general way, most of the different tetracyclic compounds were prepared on the basis of hetero-Diels−Alder reactions. Almost all the reactions involved quinoline-5,8-dione or substituted-quinoline-5,8-diones as dienophiles and 2-butenal *N*,*N*-dimethylhydrazone or 2-methoxy-2-butenal *N*,*N*-dimethylhydrazone as dienes. Generally, these reactions afforded mixtures of the corresponding diazaanthraquinones **a** and **b**, in low to moderate yields, with the last compound as the majority regioisomer.

This isomer was the sole compound isolated in four cases including addition of crotonaldehyde *N*,*N*-dimethylhydrazone to ethoxycarbonyl-3-quinoline-5,8-dione, to 2-hydroxyquinoline-5,8-dione or to 2-chloroquinoline-5,8-dione. The identification of the two regioisomers was realized both on the basis of the IR spectra of the tricyclic compounds **a** and **b** (Scheme 1) (structure **a** presented a sole carbonyl band at 1683−1702 cm^{-1} whereas structures **b** had two bands, the first one between 1651 and 1679 cm^{-1} and the second one at 1684−1705 cm^{-1}) and NMR spectra of the tetracyclic compounds (this last method was only usable for compounds unsubstituted at R_1). We have shown in a previous work[4c] a notable difference in the ^1H NMR chemical shift of the proton at position 4 (ring A). This proton was deshielded 0.4 ppm in structures **d** relative to structure **c**. For structures **c**, the chemical shift of this proton was about 8.68−8.80 ppm and 8.91−9.56 ppm in structure **d**.

* To whom correspondence should be addressed. Phone: 33 468 66 22 51, Fax: 33 468 66 22 23, e-mail:delfourn@univ-perp.fr.
† Université de Perpignan.
‡ Université Libre de Bruxelles.
§ Laboratoire Louis Lafon.

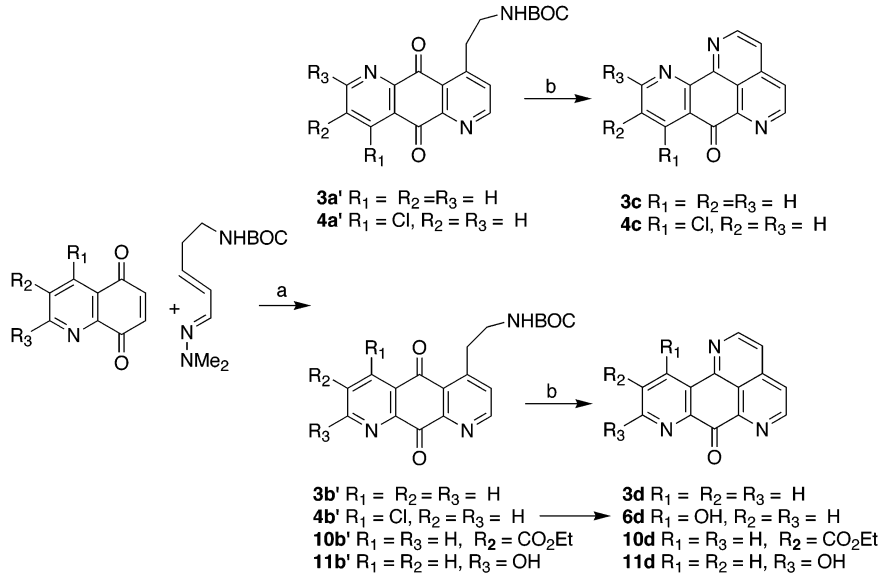
10.1021/jm0308702 CCC: $25.00
Published on Web 07/04/2003

Scheme 1[a]



[a] (a) $CHCl_3$ or CH_3CN, Ac_2O, MnO_2; (b) DMFDEA, DMF, NH_4Cl, EtOH.

Scheme 2[a]

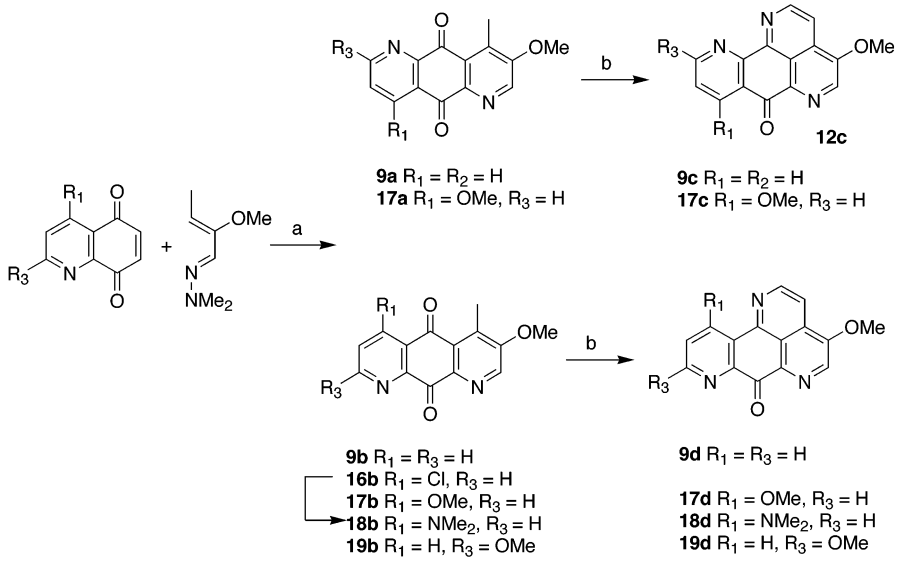


[a] (a) $CHCl_3$ or CH_3CN, Ac_2O; MnO_2; (b) TFA.

The first method to achieve the final ring D annelation was based on Bracher's methodology,[7] involving in a first step addition of dimethylformamide-diethylacetal in DMF under nitrogen and in the second step, subsequent cyclization of the formed enamine with ammonium chloride in ethanol. In some cases, these reaction conditions resulted in the formation of several secondary products. For example, it has not been possible to obtain the tetracyclic compounds **4c**, **4d**, **7c**, and **7d** from the corresponding tricyclic compounds, **4a**, **4b**, **7a**, and **7b**, respectively. Moreover, the two isomers have a high difference in reactivity of their enamine to cyclize, reactions of isomers **b** working better than those of isomers **a**, which were realized in high diluted conditions in order to decrease intermolecular reactions. A second method of formation of ring D was investigated to obtain the tetracyclic compounds which could not be synthesized according to the previous one. It was also based on a Diels−Alder strategy involving *N*-BOC-5-amino-2-penten-1-al dimethylhydrazone as the diene, the final ring D annelation being in that case realized by acid treatment (Scheme 2).

The different substituents R_1, R_2, and R_3 of ring A were introduced according to two pathways: synthesis starting from the corresponding substituted-quinoline-5,8-diones or modification of existing substituent either

Scheme 3[a]



[a] (a) $CHCl_3$, MnO_2; (b) DMFDEA, DMF, NH_4Cl, EtOH.

of the diazaanthraquinone (e.g., conversion of the nitro group of **7b** into a dimethylamino-group to give **8b**) or during the formation of ring D (e.g., compound **6d** with an hydroxyl group was obtained when the chlorodiazaanthraquinone **4b′** was reacted with TFA). It has to be noted that it has not been possible starting with the diazaanthraquinones **4a** and **4b**, using Bracher's methodology, to obtain the corresponding tetracyclic compounds **4c** and **4d**, the chloro group being substituted into a dimethylamino group. Nevertheless, compound **4c** was obtained by action of TFA on the diazaanthraquinone **4a′**. We were also interested in an attempt to design bifunctional compounds. Compound **14d** was thus obtained from the corresponding dimethoxy-tricyclic compound **14b**, derived from reaction of the dichloro derivative **13b** with sodium methylate. Direct formation of the last cycle using Bracher's methodology on this last compound gave compound **15d**.

The methoxy group at R_5 was introduced involving 2-methoxy-2-butenal *N*,*N*-dimethylhydrazone as the diene in the Diels−Alder reaction (Scheme 3). This Diels−Alder reaction was performed with four different quinoline-5,8-diones with lower yields than the same reactions with 2-butenal *N*,*N*-dimethylhydrazone.

The regioselectivity was similar with the two dienes, and the isomers **16a** and **19a** were not isolated due to the low yield of the reaction. The tricyclic compounds **9a**, **9b**, **17a**, and **19b** were transformed into the corresponding tetracyclic compound using Bracher's methodology. In these conditions, **16b** did not give the tetracyclic compound. However, **16b** was transformed into the dimethylamino derivative **18b** which led to the tetracyclic compound **18d**.

Pharmacology

In Vitro Determination of the Drug-Induced Inhibition of Human Cancer Cell Line Growth. For each of the compounds under study, six concentrations were tested on 12 distinct human cancer cell lines including various histopathological types (glioblastomas and breast, colon, lung, prostate, and bladder cancers). We made use of the colorimetric MTT assay, which indirectly assesses the effect of potentially anticancer compounds on overall growth of adherent cell lines.[8] The IC_{50} values, i.e., the concentration which reduced the mean growth value of the 12 cell lines by 50%, was determined for each drug, as compared to the mean control growth value. Table 1 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 maximun tolerated dose (MTD) index are also reported in the same table.

Discussion

The tetracyclic compounds **3c** and **3d** have been already described by Matsumoto et al.[6] and the cytotoxic activity of **3d** toward the P388 cell line was reported ($IC_{50} = 0.5\ \mu M$). Most of the synthesized compounds in this work have IC_{50} of micromolar order. Five pairs of regioisomers **c** and **d** have been tested; for three of them **3c**,**d**, **5c**,**d**, and **12c**,**d** the cytoxic activity of the two isomers was similar. For the couple **9c**,**d**, the isomer **9d** was approximatively 10 times more active than **9c** whereas for the couple **17c**,**d**, the isomer **17d** was approximatively 100 times more active than **17c**.

Among the different substituents, the methoxy group gave the more active compounds, the better position of substitution being R_5 and after R_1 and R_3. The introduction of a methoxy group both at R_5 and R_1 led to the most active compound of the series (**17d**). The activity decreased when the second methoxy group was at R_3 instead of R_1 (**19d**).

The effect of substituents on the cyctotoxicity was already studied on the two related ascididemin[4a] and meridine[4b] series. A difference in selectivity was observed between the compounds of these series and the tetracyclic compounds considered in this study. Ascididemin and meridine derivatives exhibited a low cytotoxic activity against LoVo cell line whereas pyridophenanthrolines have a normal activity. On the opposite, pyridophenanthrolines have low activity against J82 cell line, when ascididemin and meridine analogues have good activity. Despite this difference of selectivity, Matsumoto et al.[6] reported the same mechanism of

Table 1. Characterization of the in Vitro Cytotoxic-Related Antitumor Effects (IC_{50} value $\times 10^{-6}$ M) of the Compounds Listed

	cell lines (IC_{50}, μM[a])												
compounds	U-87MG	U-373MG	SW 1088	T24	J82	HCT-15	LoVo	MCF7	T-47D	A-549	A-427	PC-3	MTD (mg/kg)
3c	0.5	0.8	4	0.3	5	0.9	2	0.2	0.7	0.8	7	5	10
3d	6	4	5	0.8	5	0.6	2	0.6	0.8	3	0.7	0.9	10
4c	0.8	4	4	3	4	1	1	0.6	1	0.8	0.7	5	20
5c	4	0.8	0.9	0.7	3	0.8	0.9	0.6	0.4	0.8	0.8	0.6	10
5d	0.1	0.1	2	0.3	2	0.1	0.3	0.2	3	0.3	0.07	0.9	5
6d	8	6	6	8	6	6	5	5	6	6	6	>10	10
8d	2	1	5	1	4	2	2	0.9	4	1	0.9	4	10
9c	0.3	0.6	0.3	0.5	4	0.7	5	0.4	7	0.5	0.5	0.5	>160
9d	0.05	0.07	0.08	0.06	0.8	0.09	0.7	0.06	6	0.05	0.07	0.08	>160
10d	NT[c]												NT
11d	NT												NT
12c	6	5	5	5	5	6	>10	5	6	9	6	6	>160
12d	6	6	5	6	5	6	5	5	6	9	6	6	40
14d	5	6	5	6	5	5	4	5	5	6	5	6	40
15d	6	6	5	6	5	5	5	6	4	> 10	6	>10	40
17c	0.8	2	3	2	5	3	0.8	2	5	5	0.8	0.8	80
17d	0.008	0.007	0.008	0.009	0.2	0.04	0.008	0.03	0.06	0.005	0.006	0.04	20
18d	3	5	6	5	6	3	3	4	4	3	3	6	>160
19d	0.7	0.8	0.5	0.6	8	0.6	0.6	0.5	1	0.8	0.5	1	>160

[a] The IC_{50} value constitutes the concentration of the compound which inhibits the growth of the human cancer cells by 50% as compared to the control value. Six concentrations ranging from 10 μM 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 colorimteric assay. Data represent the mean values for three independant assays. The values for standard errors are not reported here (for the sake in clarity of the tables) because they reach less tha 3% of the mean values. [b] MTD was defined following single ip injection to B6D2F1/jico mice. [c] NT = not tested.

action for ascididemin and compound **3d**, oxidative damage to DNA via a thiol-dependent conversion of oxygen to DNA-cleaving oxygen radical.

The synthesized pyridophenanthrolines have also different toxicities depending on the substituent. The compounds sharing a methoxy group at R_5 have the lower toxicity (except **17d**). There was no clear-cut relationship between cytotoxicity against the different cell lines and toxicity; for example, **9d** has a good cytotoxicity associated with a MTD > 160 mg/kg whereas **17d**, which is the best cytotoxic compound of the series, has a relatively high toxicity (MTD = 20 mg/kg).

In conclusion, a good series of pyridophenanthrolines with high cytotoxic activity and selectivity was obtained. Experiments are under way to characterize the mechanisms of action of these drugs and to discover further antitumor activity on in vivo models.

Experimental Section

Chemistry. Chemical Synthesis. ^1H and ^{13}C NMR spectra were obtained with a JEOL 400 MHz spectrometer with the chemical shifts of 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 silica gel (15−40 μm) using the solvent systems indicated below. The purity of the different ascididemin analogues was evaluated on two analytical chromatographic systems**.** System I consisted of a inertsil ODS-3, 5 μm column (250 mm × 4.6 mm), CH_3CN/H_2O/TFA (see composition) at 1 mL/min flow rate, 260 nm, and system II consisted of a Kromasil SI, 5 μm 100 A column (250 mm × 4.6 mm), isooctane/EtOH (see composition) at 1 or 2 mL/min flow rate, 250 nm.

2-Chloro-4-methylquinoline-5,8-dione. A solution of 2-chloro-5,8-dimethoxy-4-methylquinoline[9] (2 g, 8.41 mmol) and cerium ammonium nitrate (16 g, 29.18 mmol) in a mixture CH_3CN/H_2O (140 mL/60 mL) was stirred at room temperature for 40 min. H_2O (100 mL) and a saturated solution of $NaHCO_3$ (260 mL) were added, the mixture was extracted with CH_2Cl_2 (6 × 400 mL), and the organic layers were dried over $MgSO_4$. After concentration to dryness, the expected quinone was obtained as an orange solid (1.6 g, 92%), mp 170 °C. ^1H NMR ($CDCl_3$) 2.77 (d, 3H, J = 0.7 Hz); 6.97 (d, 1H, J = 10.4 Hz) ; 7.07 (d, 1H, J = 10.4 Hz) ; 7.49 (s, 1H). ^{13}C NMR ($CDCl_3$) 21.89; 126.22 ; 131.37; 136.96; 139.50; 148.46; 153.62; 155.87; 181.91; 185.78. IR ($CHCl_3$) 1669, 1685 cm^{-1}.

***N*-BOC-1-amino-3-hydroxypropane.** To a solution of 3-amino-1-propanol (2 mL, 27 mmol) in a mixture of dioxane (60 mL), H_2O (30 mL), and 1 N NaOH (30 mL) was added at 0 °C di-*tert*-butyl dicarbonate (4.2 g, 29.7 mmol). The reaction mixture was stirred at room-temperature overnight and was acidified to pH 1 with concentrated HCl. The mixture was extracted with AcOEt (3 × 50 mL), and the organic layers were dried over $MgSO_4$ and concentrated over vacuum to give the expected product as a yellow oil (4 g, 85%). ^1H NMR ($CDCl_3$) 1.25 (s, 9H); 1.60 (m, 2H); 3.20 (m, 2H); 3.60 (m, 2H); 5.20 (br. s, 1H). ^{13}C NMR ($CDCl_3$) 156.88; 79.14; 59.08; 36.89; 32.34; 28.15.

***N*-BOC-3-aminopropanal.** A suspension of *N*-BOC-1-amino-2-hydroxypropane (18 g, 103 mmol), tetrabutylammonium chloride (2.88 g, 10.4 mmol), tetramethyl-1-piperidinyloxy (TEMPO, 1.62 g, 10.4 mmol), and *N*-chlorosuccinimide (21 g, 157.3 mmol) in a mixture 0.5 N $NaHCO_3$/0.05 N K_2CO_3 (350 mL) and $HCCl_3$ (350 mL) was vigorously stirred at room temperature for 2 h. The organic layer was recovered, dried over $MgSO_4$, and concentrated over vacuum to yield quantitatively the expected aldehyde as an orange oil. ^1H NMR ($CDCl_3$) 1.52 (s, 9H); 2.80 (m, 2H); 3.50 (m, 2H); 4.98 (br. s, 1H); 9.90 (s, 1H). ^{13}C NMR ($CDCl_3$) 23.39 (3C); 29.21; 39.69; 74.18; 150.88; 196.91.

***N*-BOC-5-amino-2-penten-1-al.** A solution of *N*-BOC-3-aminopropanal (11 g, 63.6 mmol) and formylmethylenetriphenylphosphorane (24.3 g, 80 mmol) in benzene (350 mL) was refluxed for 9 h. The solvent was evaporated over vacuum, and the crude product was first purified by flash chromatography ($HCCl_3$) to remove triphenylphosphine and then again (AcOEt/heptane 8:2) to obtain the expected product as a yellow-orange oil (3.88 g, 29%). ^1H NMR ($CDCl_3$) 1.35 (s, 9H); 2.44 (d, 2H, J = 6.8 Hz); 3.21 (m, 2H); 4.90 (br. s, 1H); 6.04 (dd, 1H, J = 8 and 15.6 Hz); 6.74 (td, 1H, J = 6.8 and 15.6 Hz); 9.39 (d, 1H, J = 8 Hz).

***N*-BOC-5-amino-2-penten-1-al dimethylhydrazone.** *N*-BOC-5-amino-2-penten-1-al (3.88 g, 19.5 mmol) was added at 0 °C to a solution of dimethylhydrazine (1.47 mL, 19.5 mmol)

and acetic acid (0.3 mL) in ether (30 mL). The reaction mixture was stirred for 10 min, and the organic layer was separated and washed with 1 N HCl and brine. After being dried over $MgSO_4$ and concentration over vacuum, the hydrazone was obtained as an orange oil (4.4 g, 94%). ^{1}H NMR ($CDCl_3$) 1.40 (s, 9H); 2.3 (m, 2H); 2.82 (m, 6H); 3.2 (m, 2H); 4.52 (br. s, 1H); 5.70 (td, 1H, J = 6.8 and 15.6 Hz); 6.22 (ddd, 1H, J = 0.8, 8.8 and 15.6 Hz); 6.96 (d, 1H, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$) 28.15; 33.05; 39.58; 42.51; 78.77; 130.84; 130.95; 135.54; 155.68.

Formation of the Diazaanthraquinone Compounds by Diels−Alder Reaction. General Method A. A mixture of dienophile, diene, and acetic anhydride in solvent was stirred. The solvent was evaporated, and the crude product was purified by flash chromatography to obtain the mixture of the two isomers. A mixture of these isomers and 85% MnO_2 in solvent was refluxed for 2 h, cooled, and filtered over Celite. The filtrate was concentrated over vacum and purified by flash chromatography to give the two expected isomers.

4-Methylpyrido[2,3-*g*]quinoline-5,10-dione (3a) and 4-Methylpyrido[3,2-*g*]quinoline-5,10-dione (3b). Method A was used and involved a mixture of quinoline-5,8-dione (0.5 g, 3.14 mmol), butenal *N*,*N*-dimethylhydrazone (0.35 g, 3.14 mmol), and acetic anhydride (0.45 mL, 4.76 mmol) in $HCCl_3$ (20 mL) which was sonicated for 1 h. After the first flash chromatography ($HCCl_3$), the mixture of the two isomers and 85% MnO_2 (1.6 g, 15.6 mmol) in $HCCl_3$ (20 mL) was refluxed for 2 h. The second flash chromatography (CH_2Cl_2/MeOH 98:2) gave the expected compounds:

(**3a**) brown solid (40 mg, 6%), mp 220 °C.^{1}H NMR ($CDCl_3$) 2.91 (s, 3H); 7.54 (d, 1H, J = 4.8 Hz); 7.75 (dd, 1H, J = 4 and 7.6 Hz); 8.67 (dd, 1H, J = 2 and 7.6 Hz); 8.91 (d, 1H, J = 4.8 Hz); 9.12 (dd, 1H, J = 2 and 4 Hz). ^{13}C NMR ($CDCl_3$) 22.75; 127.93; 128.04; 129.32; 131.50; 135.50; 148.73; 149.26; 152.11; 153.68; 155.47; 181.46; 182.87. IR ($HCCl_3$) 1689 cm^{-1}.

(**3b**) brown solid (160 mg, 23%), mp 270 °C. ^{1}H NMR ($CDCl_3$) 2.94 (s, 3H); 7.52 (d, 1H, J = 4.8 Hz); 7.76 (dd, 1H, J = 4.8 and 8.4 Hz); 8.59 (dd, 1H, J = 2 and 8.4 Hz); 8.92 (d, 1H, J = 4.8 Hz); 9.11 (dd, 1H, J = 2 and 4.8 Hz). ^{13}C NMR ($CDCl_3$) 22.81; 128.30; 128.39; 130.84; 131.55; 135.52; 147.90; 149.95; 151.74; 153.94; 155.35; 180.42; 184.02. IR ($HCCl_3$) 1672; 1700 cm^{-1}.

4-(*N*-BOC-1-aminoethane)-pyrido[2,3-*g*]quinoline-5,10-dione (3a′) and 4-(*N*-BOC-1-aminoethane)-pyrido[3,2-*g*]-quinoline-5,10-dione (3b′). Method A was used and involved a mixture of quinoline-5,8-dione (0.5 g, 3.14 mmol), *N*-BOC-5-amino-2-pentenal *N*,*N*-dimethylhydrazone (0.76 g, 3.14 mmol), and acetic anhydride (0.45 mL, 4.76 mmol) in $HCCl_3$ (20 mL) which was sonicated for 20 min. After the first flash chromatography ($HCCl_3$), the mixture of the two isomers and 85% MnO_2 (1.7 g, 16.6 mmol) in $HCCl_3$ (22 mL) was refluxed for 2 h. The second flash chromatography ($CHCl_3$) gave the expected compounds:

(**3a′**) brown solid (100 mg, 9%).^{1}H NMR ($CDCl_3$) 1.38 (s, 9H); 3.5 (m, 4H); 4.80 (br. s, 1H); 7.58 (d, 1H, J = 4.8 Hz); 7.76 (dd, 1H, J = 4.8 and 8.0 Hz); 8.69 (dd, 1H, J = 2.0 and 8.0 Hz); 8,96 (d, 1H, J = 4.8 Hz); 9.13 (dd, 1H, J = 2.0 and 4.8 Hz). ^{13}C NMR ($CDCl_3$) 28.43; 35.85; 40.63; 79.67; 128.46; 129.51; 129.71; 131.88; 135.91; 149.04; 149.90; 153.04; 154.25; 155.84; 156.21; 181.57; 183.4. IR ($HCCl_3$) 1690 cm^{-1}.

(**3b′**) brown solid (100 mg, 9%). ^{1}H NMR ($CDCl_3$) 1.35 (s, 9H); 3.46 (m, 4H); 4.74 (br. s 1H); 7.54 (d, 1H, J = 4.8 Hz); 7.77 (dd, 1H, J = 4.8 and 8.0 Hz); 8.60 (dd, 1H, J = 1.2 and 8.0 Hz); 8.97 (d, 1H, J = 4.8 Hz); 9.14 (dd, 1H, J = 1.2 and 4.8 Hz). ^{13}C NMR ($CDCl_3$) 28.30; 35.52; 40.21; 79.51; 128.33; 128.43; 130.90; 131.48; 135.65; 147.83; 150.19; 152.31; 153.97; 155.42; 155.84; 180.20; 184.10. IR ($HCCl_3$) 1672; 1676; 1702 cm^{-1}.

4-Chloro-9-methylpyrido[2,3-*g*]quinoline-5,10-dione (4a) and 4-Chloro-6-methylpyrido[3,2-*g*]quinoline-5,10-dione (4b). Method A was used and involved a mixture of 4-chloroquinoline-5,8-dione (2 g, 10.32 mmol), butenal *N*,*N*-dimethylhydrazone (1.16 g, 10.32 mmol), and acetic anhydride (1.46 mL, 15.48 mmol) in $HCCl_3$ (28 mL) which was sonicated for 1 h. After the first flash chromatography (CH_2Cl_2/MeOH 98:2) the mixture of the two isomers and 85% MnO_2 (12.3 g, 120.3 mmol) in $HCCl_3$ (140 mL) was refluxed for 3 h. The second flash chromatography (CH_2Cl_2/MeOH 98:2) gave the expected compounds:

(**4a**) brown solid (200 mg, 8%).^{1}H NMR ($CDCl_3$) 2.90 (s, 3H); 7.54 (d, 1H, J = 4.8 Hz); 7.74 (d, 1H, J = 4.8 Hz); 8.92 (d, 1H, J = 4.8 Hz); 8.93 (d, 1H, J = 4.8 Hz). ^{13}C NMR ($CDCl_3$) 22.50; 126.83; 128.12; 130.78; 131.22; 145.78; 150.11; 151.00; 151.79; 154.04; 154.17; 180.05; 182.20. IR ($HCCl_3$) 1684 cm^{-1}.

(**4b**) brown solid (500 mg, 19%), mp 216 °C. ^{1}H NMR ($CDCl_3$) 2.85 (s, 3H); 7.54 (d, 1H, J = 4.8 Hz); 7.74 (d, 1H, J = 5.2 Hz); 8.91 (d, 1H, J = 4.8 Hz); 8.91 (d, 1H, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$) 22.67; 128.35; 130.09; 131.41; 131.99; 145.49; 148.84; 150.02; 151.54; 154.10; 154.14; 180.13; 183.09. IR ($HCCl_3$) 1678; 1696 cm^{-1}.

4-Chloro-9-(*N*-BOC-1-aminoethane)-5,10-dihydropyrido-[2,3-*g*]quinoline-5,10-dione (4a′) and 4-Chloro-6-(*N*-BOC-1-aminoethane)-5,10-dihydropyrido[3,2-*g*]quinoline-5,10-dione (4b′). Method A was used and involved a mixture of 4-chloroquinoline-5,8-dione (0.6 g, 3.1 mmol), *N*-BOC-5-amino-2-pentenal *N*,*N*-dimethylhydrazone (0.75 g, 3.1 mmol), and acetic anhydride (0.45 mL, 4.76 mmol) in $HCCl_3$ (8.5 mL) which was sonicated for 30 min. After concentration, the mixture and 85% MnO_2 (2.7 g, 26.4 mmol) in $HCCl_3$ (22 mL) was refluxed for 2 h. The second flash chromatography (CH_2Cl_2/MeOH 99:1) gave the expected compounds:

(**4a′**) brown solid (70 mg, 6%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 1.35 (s, 9H); 3.45−3.52 (m, 4H); 4.86 (br. s, 1H); 7.56 (d, 1H, J = 4.0 Hz); 7.74 (d, 1H, J = 5.2 Hz); 8.90 (d, 1H, J = 5.2 Hz); 8.94 (d, 1H, J = 4.0 Hz). ^{13}C NMR ($CDCl_3$) 28.37; 35.32; 40.30; 79.47; 126.84; 128.04; 130.88; 131.17; 145.78; 150.34; 150.98; 152.29; 154.05; 154.36; 155.88; 179.76; 182.32. IR ($HCCl_3$) 1695 cm^{-1}.

(**4b′**) brown solid (200 mg, 17%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 1.35 (s, 9H); 3.4 (m, 2H); 3.51 (m, 2H); 4.86 (br. s, 1H); 7.57 (d, 1H, J = 4.8 Hz); 7.75 (d, 1H, J = 5.2 Hz); 8.91 (d, 1H, J = 4.8 Hz); 8.95 (d, 1H, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$) 28.24; 34.96; 40.33; 79.47; 128.46; 130.15; 131.06; 131.59; 145.20; 148.76; 149.71; 151.74; 153.88; 153.92; 155.84; 179.76; 183.20. IR ($HCCl_3$) 1705 cm^{-1}.

4-Methoxy-9-methylpyrido[2,3-*g*]quinoline-5,10-dione (5a) and 4-Methoxy-6-methylpyrido[3,2-*g*]quinoline-5,10-dione (5b). Method A was used and involved a mixture of 4-methoxyquinoline-5,8-dione (0.5 g, 2.65 mmol), butenal *N*,*N*-dimethylhydrazone (0.32 g, 2.86 mmol), and acetic anhydride (0.4 mL, 4.23 mmol) in $HCCl_3$ (8 mL) which was refluxed for 1 h. After the first flash chromatography (CH_2Cl_2/MeOH 98:2), the mixture of the two isomers and 85% MnO_2 (2.3 g, 22.48 mmol) in $HCCl_3$ (26 mL) was refluxed for 2 h. The second flash chromatography (CH_2Cl_2/MeOH 98:2) gave the expected compounds:

(**5a**) red solid (57 mg 9%).^{1}H NMR ($CDCl_3$) 2.84 (s, 3H); 4.06 (s, 3H); 7.18 (d, 1H, J = 6 Hz); 7.46 (d, 1H, J = 4.4 Hz); 8.871 (d, 1H, J = 6 Hz); 8.874 (d, 1H, J = 4.4 Hz). IR ($HCCl_3$) 1683 cm^{-1}.

(**5b**) orange solid (293 mg, 44%). ^{1}H NMR ($CDCl_3$) 2.80 (s, 3H); 4.05 (s, 3H); 7.2 (d, 1H, J = 6.0 Hz); 7.48 (d, 1H, J = 4.8 Hz); 8.85 (d, 1H, J = 6.0 Hz); 8.88 (d, 1H, J = 4.8 Hz). ^{13}C NMR ($CDCl_3$) 21.75; 43.41; 112.74; 119.72; 130.93; 131.04; 148.32; 149.22; 150.26; 151.60; 152.80; 155.11; 181.44; 184.53. IR ($HCCl_3$) 1675; 1700 cm^{-1}.

4-Nitro-9-methylpyrido[2,3-*g*]quinoline-5,10-dione (7a) and 4-Nitro-6-methylpyrido[3,2-*g*]quinoline-5,10-dione (7b). Method A was used and involved a mixture of 4-nitroquinoline-5,8-dione (0.8 g, 3.92 mmol), butenal *N*,*N*-dimethylhydrazone (0.65 g, 5.8 mmol), and acetic anhydride (0.55 mL, 5.8 mmol) in $HCCl_3$ (8 mL) which was sonicated for 30 min. After the first flash chromatography (CH_2Cl_2/MeOH 98:2), the mixture of the two isomers and MnO_2 (2.9 g, 28.3 mmol) in $HCCl_3$ (29 mL) was refluxed for 2 h. The second flash chromatography (CH_2Cl_2/MeOH 98:2) gave the expected compounds:

(**7a**) yellow solid (110 mg, 11%).^{1}H NMR ($CDCl_3$) 2.98 (s, 3H); 7.19 (d, 1H, J = 5.6 Hz); 7.54 (d, 1H, J = 4.8 Hz); 8.79 (d, 1H, J = 5.6 Hz); 8.94 (d, 1H, J = 4.8 Hz). IR ($HCCl_3$) 1703 cm^{-1}.

(**7b**) yellow solid (165 mg, 16%). ^{1}H NMR ($CDCl_3$) 2.85 (s, 3H); 7.6 (d, 1H, J = 4.8 Hz); 7.74 (d, 1H, J = 4.8 Hz); 8.99 (d, 1H, J = 4.8 Hz); 9.33 (d, 1H, J = 4.8 Hz).

4-(Dimethylamino)-6-methylpyrido[3,2-*g*]quinoline-5,10-dione (8b). A solution of compound **7b** (150 mg, 0.558 mmol) and *N*,*N*-dimethylformamide diethyl acetal (DMF-DEA, 0.4 mL, 1.95 mmol) in (2.1 mL) was warmed at 130 °C for 1 h. After concentration of the solvent over vacuum, the expected dimethylamino derivative was obtained and used without further purification in the next step (140 mg, 94%). ^{1}H NMR ($CDCl_3$) 2.77 (s, 3H); 3.05 (s, 6H); 6.89 (d, 1H, 6.0 Hz); 7.39 (d, 1H, 4.8 Hz); 8.42 (d, 1H, 6.0 Hz); 8.74 (d, 1H, J = 4.8 Hz).

3-Methoxy-4-methylpyrido[2,3-*g*]quinoline-5,10-dione (9a) and 3-Methoxy-4-methylpyrido[3,2-*g*]quinoline-5,10-dione (9b). Method A was used and involved a mixture of quinoline-5,8-dione (1 g, 6.29 mmol) and 2-methoxy-2-butenal *N*,*N*-dimethylhydrazone[10] (1.78 g, 12.57 mmol) in $HCCl_3$ (25 mL) which was stirred at room temperature for 5 h. After the first flash chromatography (CH_2Cl_2/MeOH 95:5), the mixture of the two isomers and MnO_2 (1 g, 9.8 mmol) in $HCCl_3$ (30 mL) was stirred at room temperature for 1 h. The second flash chromatography (CH_2Cl_2/MeOH 99:1) gave the expected compounds:

(**9a**) brown solid (110 mg, 7%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 2.79 (s, 3H); 4.11 (s, 3H); 7.72 (dd, 1H, J = 4.8 and 8.1 Hz); 8.66 (s, 1H); 8.67 (dd, 1H, J = 8.1 and 1.9 Hz); 9.10 (dd, 1H, J = 4.8 and 1.9 Hz). ^{13}C NMR ($CDCl_3$) 13.03; 56.87; 127.88; 129.50; 129.95; 135.50; 136.64; 139.26; 142.56; 149.33; 155.11; 157.24; 180.63; 183.56. IR ($HCCl_3$) 1688 cm^{-1}.

(**9b**) brown solid (190 mg, 12%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 2.77 (s, 3H); 4.12 (s, 3H); 7.74 (dd, 1H, J = 4.6 and 8.0 Hz); 8.60 (dd, 1H, J = 8.0 and 1.6 Hz); 8.68 (s, 1H); 9.12 (dd, 1H, J = 4.6 and 1.6 Hz). ^{13}C NMR ($CDCl_3$) 12.98; 56.93; 127.99; 129.06; 131.27; 135.53; 136.84; 138.81; 143.27; 148.16; 155.20; 157.16, 179.69; 184.59. IR ($HCCl_3$) 1670; 1692 cm^{-1}.

3-Ethoxycarbonyl-6-(2′-*N*-BOC-aminoethyl)pyrido[3,2-*g*]quinoline-5,10-dione (10b′). Method A was used and involved a mixture of 3-ethylquinolinecarboxylate-5,8-dione (1.05 g, 4.54 mmol), *N*-BOC-5-amino-2-penten-1-al *N*,*N*-dimethylhydrazone (1.1 g, 4.56 mmol), and acetic anhydride (0.44 mL, 4.6 mmol) in acetonitrile (15 mL) which was stirred at room temperature for 24 h. After concentration, the mixture and MnO_2 (5 g, 48.9 mmol) in $HCCl_3$ (150 mL) was refluxed for 1.5 h. The second flash chromatography (CH_2Cl_2/MeOH 99:1) gave the expected compound as a brown solid (60 mg, 3%), mp 170 °C. ^{1}H NMR ($CDCl_3$) 1.36 (s, 9H); 1.47 (t, 3H, J = 7.4 Hz); 3,52 (m, 4H); 4.51 (q, 2H, J = 7.4 Hz); 4.78 (br. s, 1H); 7.57 (d, 1H, J = 5.2 Hz); 8.99 (d, 1H, J = 5.2 Hz); 9.17 (d, 1H, J = 2.2 Hz); 9.64 (d, 1H, J = 2.2 Hz). ^{13}C NMR ($CDCl_3$) 14.33; 28.40; 35.74; 40.22; 62.62; 79.63; 128.65; 130.33; 130.49; 131.83; 137.30; 149.60; 150.23; 152.72; 154.23; 155.72; 155.98; 163.52; 179.69; 183.38. IR ($CHCl_3$) 3457; 1726; 1705; 1677 cm^{-1}.

2-Hydroxy-6-methylpyrido[3,2-*g*]quinoline-5,10-dione (11b). Method A was used and involved a mixture of 5,8-dioxocarbostyril (1 g, 5.71 mmol), 2-butenal *N*,*N*-dimethylhydrazone (0.703 g, 6.28 mmol), and acetic anhydride (6.2 mL, 65.71 mmol) in acetonitrile (220 mL) which was refluxed for 6 h. After the first flash chromatography (CH_2Cl_2/MeOH 98:2), 85% MnO_2 (3 g, 29.3 mmol) and $HCCl_3$ (75 mL) were added, and the mixture was stirred overnight at room temperature. The second flash chromatography (CH_2Cl_2/MeOH 99:1) gave the expected compound as a beige solid (160 mg, 12%), mp > 260 °C. ^{1}H NMR (DMSO-d_6) 2.79 (s, 3H); 6.82 (d, 1H, J = 9.5 Hz); 7.73 (d, 1H, J = 5.2 Hz); 8.05 (d, 1H, J = 9.5 Hz); 8.85 (d, 1H, J = 5.2 Hz); 12.27 (br. s, 1H). ^{13}C NMR (DMSO-d_6) 21.92; 114.30; 122.66; 127.30; 131.52; 135.94; 148.60; 149.80; 152.48 (2C); 176.41; 182.13 (2C). IR ($CHCl_3$) 1684; 1664 cm^{-1}.

2-Hydroxy-6-(2′-*N*-Boc-aminoethyl)pyrido[3,2-*g*]quinoline-5,10-dione(11b′). Method A was used and involved a mixture of 5,8-dioxocarbostyril (0.98 g, 5.59 mmol), *N*-BOC-5-amino-2-penten-1-al *N*,*N*-dimethylhydrazone (1.49 g, 6.15 mmol), and acetic anhydride (5.8 mL, 61.5 mmol) in acetonitrile (30 mL) which was stirred at room temperature for 16 h. After concentration, the mixture and 85% MnO_2 (7 g, 68.4 mmol) in $HCCl_3$ (180 mL) was refluxed for 1.5 h. Purification on flash chromatography (CH_2Cl_2/MeOH 98:2) gave the expected compound as a brown solid (230 mg, 12%), mp 252 °C. ^{1}H NMR ($CDCl_3$) 1.36 (s, 9H); 3.49 (m, 4H); 4.73 (br. s, 1H); 6.94 (d, 1H, J = 9.6 Hz); 7.54 (d, 1H, J = 4.8 Hz); 8.10 (d, 1H, J = 9.6 Hz); 8.89 (d, 1H, J = 4.8 Hz); 9.66 (br. s, 1H). ^{13}C NMR ($CDCl_3$) 28.29 (3C); 35.53; 40.24; 77.8; 117.01; 127.87; 128.62; 132.29; 136.18; 138.04; 148.25; 152.26; 153.33; 155.86; 176.36; 181.35. IR ($CHCl_3$) 3457; 3340; 1693; 1663 cm^{-1}.

2-Methoxy-6-methylpyrido[2,3-*g*]quinoline-5,10-dione (12a) and 2-Methoxy-6-methylpyrido[3,2-*g*]quinoline-5,10-dione (12b). Method A was used and involved a mixture of 2-methoxyquinoline-5,8-dione (1.3 g, 6.88 mmol), 2-butenal *N*,*N*-dimethylhydrazone (0.81 g, 7.22 mmol), and acetic anhydride (7.5 mL, 79.5 mmol) in acetonitrile (150 mL) which was stirred at room temperature for 68 h. MnO_2 (85%) (7 g, 68.4 mmol) was added, and the mixture was stirred at room temperature for 6 h. Purification on flash chromatography (CH_2Cl_2/MeOH 99:1) gave the expected compounds:

(**12a**) brown solid (100 mg, 6%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 2.92 (s, 3H); 4.20 (s, 3H); 7.13 (d, 1H, J = 8.8 Hz); 7.50 (d, 1H, J = 5.0 Hz); 8.53 (d, 1H, J = 8.8 Hz); 8.90 (d, 1H, J = 5.0 Hz). ^{13}C NMR ($CDCl_3$) 22.80; 54.72; 117.30; 124.89; 128.96; 131.22; 137.91; 148.23; 149.40; 151.76; 153.55; 167.66; 180.92; 183.24. IR ($CHCl_3$) 1685, 1603 cm^{-1}.

(**12b**) brown solid (550 mg, 32%), mp 128 °C. ^{1}H NMR ($CDCl_3$) 2.89 (s, 3H); 4.14 (s, 3H); 7.07 (d, 1H, J = 8.8 Hz); 7.44 (d, 1H, J = 4.8 Hz); 8.37 (d, 1H, J = 8.8 Hz); 8.85 (d, 1H, J = 4.8 Hz). ^{13}C NMR ($CDCl_3$) 29.69; 54.92; 117.58; 126.24; 128.09; 131.30; 137.73; 147.31; 150.00; 151.34; 153.38; 167.39; 180.44; 183.70. IR ($CHCl_3$) 1698; 1667; 1603 cm^{-1}.

2,4-Dichloro-6-methylpyrido[3,2-*g*]quinoline-5,10-dione (13b). Method A was used and involved a mixture of 2,4-chloroquinoline-5,8-dione (0.6 g, 2.63 mmol), 2-butenal dimethylhydrazone (0.325 g, 2.89 mmol), and acetic anhydride (5 mL) in CH_3CN (120 mL) which was stirred at room temperature under nitrogen atmosphere, in the dark for 20 h. After concentration, the crude product and MnO_2 (85%) (3.65 g, 35.7 mmol) in $CHCl_3$ (140 mL) was stirred at room temperature for 56 h. Flash chromatography (CH_2Cl_2 gave the product as a brown solid (314 mg, 41%), mp 177 °C. ^{1}H NMR ($CDCl_3$) 2.87 (s, 3H); 7.56 (d, 1H, J = 4.8 Hz); 7.79 (s, 1H); 8.93 (d, 1H, J = 4.8 Hz). ^{13}C NMR ($CDCl_3$) 22.41; 125.44; 127.84; 131.13; 131.30; 147.44; 149.81; 150.62; 151.90; 154.30; 156.58; 179.12; 180.66. IR ($CHCl_3$) 1706; 1683 cm^{-1}.

2,4-Dimethoxy-6-methylpyrido[3,2-*g*]quinoline-5,10-dione (14b). A mixture of compound **13b** (80 mg, 0.27 mmol) and sodium methoxyde (300 mg Na/40 mL MeOH; 13.04 mmol) in MeOH (40 mL) was refluxed for 17 h. After concentration, H_2O (50 mL) was added, and the mixture was neutralized with 25% HCl and extracted with CH_2Cl_2 (3 × 50 mL). The organic layers were dried over $MgSO_4$, and the solvent was removed over vacuum to yield quantitatively the expected compound as a brown solid, mp 219 °C. ^{1}H NMR ($CDCl_3$) 2.88 (s, 3H); 4.03 (s, 3H); 4.07 (s, 3H); 6.53 (s, 1H), 7.45 (d, 1H, J = 4.8 Hz); 8.83 (d, 1H, J = 4.8 Hz). ^{13}C NMR ($CDCl_3$) 22.64; 54.73; 56.80; 97.79; 117.61; 129.55; 131.46; 148.67; 149.41; 150.73; 152.96; 167.95; 168.00; 180.91; 183.41. IR ($CHCl_3$) 1701; 1668 cm^{-1}.

3-Methoxy-4-methyl-6-chloropyrido[3,2-*g*]quinoline-5,10-dione (16b). Method A was used and involved a mixture of chloroquinolinedione (1.37 g, 7.1 mmol) and 2-methoxy-2-butenal dimethylhydrazone (1 g, 7.05 mmol) in $CHCl_3$ (45 mL) which was stirred at room temperature in the dark for 5.5 h. After the first flash chromatography, $CHCl_3$/MeOH 98:2), the major isomer and 85% MnO_2 (1 g, 9.78 mmol) in $CHCl_3$ (30 mL) was stirred at room temperature for 1 h. The second flash chromatography ($CHCl_3$/MeOH 97:3) gave the product as a yellow solid (100 mg, 5%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 2.68

(s, 3H); 4.11 (s, 3H); 7.71 (d, 1H, J = 5.2 Hz); 8.64 (s, 1H); 8.90 (d, 1H, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$) 12.96; 56.97; 128.92; 130.72; 130.98; 136.95; 138.12; 141.93; 145.06; 150.21; 153.85; 157.55; 179.31; 183.67. IR ($CHCl_3$) 1696; 1684 cm^{-1}.

3,9-Dimethoxy-4-methylpyrido[2,3-*g*]quinoline-5,10-dione (17a, tricycle73) and 3,6-Dimethoxy-4-methylpyrido-[3,2-*g*]quinoline-5,10-dione (17b). Method A was used and involved a mixture of 4-methoxyquinoline-5,8-dione (6.0 g, 31.75 mmol) and 2-methoxy-2-butenal dimethylhydrazone (6.75 g, 47.53 mmol) in $CHCl_3$ (210 mL) which was stirred at room temperature under nitrogen atmosphere, in the dark, for 16 h. After the first flash chromatography $CHCl_3$/MeOH 95:5), the mixture of the two isomers and 85% MnO_2 (29 g, 283.5 mmol) in $CHCl_3$ (30 mL) was stirred at room temperature for 1.5 h. The second flash chromatography ($CHCl_3$/MeOH 97:3) gave the expected products:

(**17a**) yellow solid (245 mg, 3%), mp> 260 °C. ^{1}H NMR ($CDCl_3$) 2.73 (s, 3H); 4.08 (s, 3H); 4.13 (s, 3H); 7.16 (d, 1H, J = 5.9 Hz); 8.62 (s, 1H); 8.86 (d, 1H, J = 5.9 Hz). ^{13}C NMR ($CDCl_3$) 12.99; 56.89; 56.99; 111.07; 119.54; 128.56; 136.90; 138.40; 143.56; 152.0; 155.52; 156.80; 166.53; 180.24; 184.22. IR ($CHCl_3$) 1687 cm^{-1}.

(**17b**) brown solid (992 mg, 11%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 2.68 (s, 3H); 4.09 (s, 3H); 4.10 (s, 3H); 7.18 (d, 1H, J = 5.5 Hz); 8.60 (s, 1H); 8.88 (d, 1H, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$) 12.85; 56.81; 56.84; 111.14; 121.32; 130.95; 136.43; 137.79; 141.95; 150.31; 155.44; 157.33; 165.97; 180.13; 184.24. IR ($CHCl_3$) 1678, 1692 cm^{-1}.

3-Methoxy-4-methyl-6-dimethylaminopyrido[3,2-*g*]quinoline-5,10-dione (18b). A solution of compound **16b** (90 mg, 0.31 mmol), dimethylamine hydrochloride (127 mg, 1.56 mmol), and NaOH (63 mg, 1.56 mmol) in THF/H_2O (4 mL:2 mL) was refluxed for 1 h. After concentration, a mixture of CH_2Cl_2/MeOH 95:5 (50 mL) was added to the crude product. The organic layer was separated and dried over $MgSO_4$. After concentration, the crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to give the compound as a yellow solid (80 mg, 87%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 2.64 (s, 3H); 3.06 (s, 6H); 4.08 (s, 3H); 6.95 (d, 1H, J = 5.9 Hz); 8.53 (d, 1H, J = 5.9 Hz); 8.56 (s, 1H). ^{13}C NMR ($CDCl_3$) 12.62; 43.40; 56.80; 112.39; 120.50; 132.23; 135.90; 136.08; 141.86; 150.53; 151.70; 155.04; 157.19; 180.67; 185.45. IR ($CHCl_3$) 1693; 1654 cm^{-1}.

2,7-Dimethoxy-6-methylpyrido[3,2-*g*]quinoline-5,10-dione (19b). Method A was used and involved a mixture of 2-methoxyquinoline-5,8-dione (1.0 g, 5.3 mmol) and 2-methoxy-2-butenal dimethylhydrazone (0.75 g, 5.3 mmol) in THF (70 mL) which was stirred at room temperature under nitrogen atmosphere, in the dark for 40 h. After concentration the crude product and 85% MnO_2 (5.4 g, 53 mmol) in $CHCl_3$ (80 mL) was stirred at room temperature for 2 h. Flash chromatography ($CHCl_3$) gave the product as a brown solid (120 mg, 8%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 2.74 (s, 3H); 4.09 (s, 3H); 4.20 (s, 3H); 7.09 (d, 1H, J = 8.4 Hz); 8.41 (d, 1H, J = 8.4 Hz); 8.63 (s, 1H). ^{13}C NMR ($CDCl_3$) 54.41; 57.43 (2C); 116.44; 125.13; 125.62; 129.64; 137.76; 139.53; 142.19; 148.19; 153.58; 165.72; 166.67; 177.99; 181.13. IR ($CHCl_3$) 1693; 1667 cm^{-1}.

Formation of the Tetracyclic Compounds. General Method B. A mixture of diazaantraquinone and dimethylformamide diethylacetal in DMF was warmed at 120 °C under nitrogen atmosphere for 1 h. After concentration of the solvent, NH_4Cl and solvent were added, and the reaction media was refluxed. The solvent was removed, H_2O was added, and the mixture was extracted with CH_2Cl_2. The organic layers were dried over $MgSO_4$, and the crude product was purified to give the expected tetracyclic compound. **General Method C.** A mixture of diazaanthraquinone and trifluoroacetic acid was stirred at room temperature. TFA was removed over vacum, and $NaHCO_3$ saturated solution and CH_2Cl_2/MeOH 95:5 were added. The organic layer was recovered and dried over $MgSO_4$. The solvent was removed over vacuum to yield the tetracyclic compound.

7*H*-Pyrido[4,3,2-*de*][1,10]phenanthrolin-7-one (3c). Method B was used and involved a mixture of compound **3a** (0.63 g, 2.70 mmol) and DMF−DEA (1.7 mL, 9.91 mmol) in DMF (4.5 mL) which was warmed at 120 °C, under nitrogen atmosphere for 1 h. After concentration, NH_4Cl (3.5 g, 65.4 mmol) and ethanol (60 mL) were added, and the mixture was refluxed for 30 min. After concentration, H_2O (50 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). After drying, the organic layers were concentrated to give the expected tetracyclic compound as a green solid (0.6 g, 95%), mp 240 °C. ^{1}H NMR ($CDCl_3$) 7.68 (dd, 1H, J = 4.4 and 8.0 Hz); 7.87 (d, 1H, J = 5.6 Hz); 8.02 (d, 1H, J = 5.2 Hz); 8.77 (dd, 1H, J = 1.6 and 8.0 Hz); 9.11 (d, 1H, J = 5.2 Hz); 9.16 (dd, 1H, J = 1.6 and 4.4 Hz); 9.19 (d, 1H, J = 5.6 Hz). ^{13}C NMR ($CDCl_3$) 120.95; 124.40; 126.14; 129.32; 136.78; 139.09; 147.45; 148.58; 148.82; 148.96; 150.66; 152.00; 155.73; 181.96. IR ($CHCl_3$) 1681 cm^{-1}. MS m/z 233 (96); 205 (100); 178 (29). t_R is 5.41 min (98.7% purity), using system I (CH_3CN/H_2O/TFA 20:80:0.1), and t_R is 7.85 min (97.3% purity), using system II (isooctane/EtOH 70:30), flow rate 2 mL/min.

7*H*-Pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (3d). Method B was used and involved a mixture of compound **3b** (0.87 g, 3.88 mmol), DMF-DEA (2.5 mL, 14.6 mmol) in DMF (6.1 mL) which was warmed at 120 °C, under nitrogen atmosphere for 1 h. After concentration, NH_4Cl (4.9 g, 91.6 mmol) and ethanol (780 mL) were added and the mixture was refluxed for 30 min. After concentration H_2O (50 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). After drying, the organic layers were concentrated to give the expected tetracyclic compound as a yellow solid (0.72 g, 80%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 7.76 (dd, 1H, J = 4.4 and 8.0 Hz); 7.80 (d, 1H, J = 5.2 Hz); 7.99 (d, 1H, J = 5.6 Hz); 8.93 (d, 1H, J = 5.6 Hz); 9.05 (dd, 1H, J = 1.6 and 4.4 Hz); 9.17 (dd, 1H, J = 1.6 and 8.0 Hz); 9.19 (d, 1H, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$) 119.39; 120.01; 123.85; 128.15; 132.87; 133.80; 138.65; 147.54; 147.74; 148.93; 149.49; 149.99; 152.97; 180.73. IR ($CHCl_3$) 1693 cm^{-1}. MS m/z 233 (100); 205 (68); 178 (56); 151 (42). t_R is 9.11 min (92% purity), using system I (CH_3CN/H_2O/TFA 20:80:0.1), and t_R is 10.50 min (100% purity), using system II (isooctane/EtOH 80:20), Flow rate 2 mL/min.

8-Chloro-7*H*-pyrido[4,3,2-*de*][1,10]phenanthrolin-7-one (4c). Method C was used and involved a mixture of compound **4a′** (260 mg, 0.67 mmol), and TFA (2.6 mL) which was stirred for 64 h. After evaporation of TFA, CH_2Cl_2/MeOH 95:5 (200 mL) and Na_2CO_3 (50 mL) were added. The residue was washed with ether to yield the tetracyclic compound as a brown solid (40 mg, 28%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 7.68 (d, 1H, J = 5.2 Hz); 7.89 (d, 1H, J = 5.5 Hz); 8.01 (d, 1H, J = 5.5 Hz); 8.96 (d, 1H, J = 5.2 Hz); 9.14 (d, 1H, J = 5.5 Hz); 9.19 (d, 1H, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$) 119.87; 120.88; 123.61; 126.31; 129.01; 138.56; 146.87; 147.37; 148.46; 148.94; 149.76; 153.85; 153.96; 179.87. MS (m/z) 268 (17); 266 (37); 240 (19); 239 (65); 238 (41); 204 (100). t_R is 5.22 min (99.9% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 15.15 min (99.9% purity), using system II (isooctane/EtOH 80: 20), flow rate 2 mL/min.

8-Methoxy-7*H*-pyrido[4,3,2-*de*][1,10]phenanthrolin-7-one (5c). Method B was used and involved a mixture of compound **5a** (0.74 g, 2.91 mmol) and DMF−DEA (2 mL, 11.7 mmol) in DMF (5.2 mL) which was warmed at 120 °C, under nitrogen atmosphere for 1 h. After concentration, NH_4Cl (4.5 g, 84.1 mmol) and ethanol (67 mL) were added, and the mixture was refluxed for 30 min. After concentration, H_2O (50 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). After drying, the organic layers were concentrated, and the crude product was purified by flash chromatography ($HCCl_3$/MeOH 98:2) to give the expected tetracyclic compound as an orange solid (0.28 g, 37%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 4.20 (s, 3H); 7.13 (d, 1H, J = 5.6 Hz); 7.82 (d, 1H, J = 5.2 Hz); 7.94 (d, 1H, J = 6.0 Hz); 8.92 (d, 1H, J = 5.6 Hz); 9,07 (d, 1H, J = 6.0 Hz); 9.13 (d, 1H, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$) 56.77; 109.26; 119.08; 119.70; 120.47; 123.09; 138.50; 147.85; 148.25; 148.69; 150.66; 154.08; 155.68; 167.54; 180.40. IR ($CHCl_3$) 1677 cm^{-1}. MS m/z 263 (89); 262 (100); 234 (40); 233 (38); 206 (30); 205 (75). t_R is 3.75 min (99.7% purity), using system I (CH_3CN/H_2O/TFA 20:80:0.1), and t_R is 11.69

min (97.0%purity), using system II (isooctane/EtOH 60:40), flow rate 2 mL/min.

11-Methoxy-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (5d). Method B was used and involved a mixture of compound **5b** (1.14 g, 4.48 mmol) and DMF−DEA (3 mL, 17.5 mmol) in DMF (8 mL) which was warmed at 120 °C, under nitrogen atmosphere for 1 h. After concentration, NH_4Cl (4.5 g, 84.1 mmol) and ethanol (67 mL) were added, and the mixture was refluxed for 30 min. After concentration, H_2O (50 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). After drying, the organic layers were concentrated, and the crude product was purified by flash chromatography ($HCCl_3$/MeOH 98:2) to give the expected tetracyclic compound as a yellow solid (0.59 g, 50%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 4.15 (s, 3H); 7.26(d, 1H, J = 6.0 Hz); 7.70 (d, 1H, J = 6.0 Hz); 7.96 (d, 1H, J = 5.6 Hz); 8.85 (d, 1H, J = 6.0 Hz); 8.97 (d, 1H, J = 6.0 Hz); 9.15 (d, 1H, J = 5.6 Hz). ^{13}C NMR ($CDCl_3$) 57.05; 111.33; 118.72; 119.61; 122.12; 124.29; 138.56; 146.71; 147.10; 148.69; 149.81; 150.96; 153.13; 165.83; 180.82. IR ($CHCl_3$) 1691 cm^{-1}. MS *m*/*z* 263 (87); 262 (100); 234 (28); 205 (69). t_R is 3.76 min (99.1% purity), using system I (CH_3CN/H_2O/TFA 20:80:0.1), and t_R is 22.66 min (99.1% purity), 11.69 min using system II (isooctane/EtOH 60:40), flow rate 2 mL/min.

11-Hydroxy-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (6d). Method C was used and involved a mixture of compound **4b′** (50 mg, 0.129 mmol), and TFA (0.5 mL) which was stirred for 24 h. After evaporation of TFA, CH_2Cl_2 (10 mL) and $NaHCO_3$ (until pH 10) were added. Concentration of the organic layer gave the expected tetracyclic as a yellow solid (20 mg, 63%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 7.20 (d, 1H, J = 5.6 Hz); 7.83 (d, 1H, J = 6.0 Hz); 8.00 (d, 1H, J = 6.0 Hz); 8.72 (d, 1H, J = 6.0 Hz); 8.76 (d, 1H, J = 6.0 Hz); 9.24 (d, 1H, J = 5.6 Hz), 14.65 (s, 1H). ^{13}C NMR (DMSO-d_6) 116.47; 117.05; 118.90; 119.74; 123.74; 138.58; 143.86; 14.92; 14.73; 15.15; 15.55; 16.78; 180.29. t_R is 4.81 min (98.3% purity), using system I (CH_3CN/H_2O/TFA 10:90:0.1), and t_R is 15.57 min (96.1% purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

11-(Dimethylamino)-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (8d). Method B was used and involved a mixture of compound **8b** (80 mg, 0.3 mmol), DMF-DEA (0.21 mL, 1.22 mmol) in DMF (1.2 mL) which was warmed at 120 °C, under nitrogen atmosphere for 1 h. After concentration, NH_4Cl (0.5 g, 9.3 mmol) and ethanol (80 mL) were added, and the mixture was refluxed for 40 min. After concentration H_2O (5 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). After drying, the organic layers were concentrated to give quantitatively the expected tetracyclic compound as a red solid, which decomposes before melting. ^{1}H NMR ($CDCl_3$) 3.00 (s, 6H); 7.09 (d, 1H, J = 5.2 Hz); 7.57 (d, 1H, J = 5.6 Hz); 7.90 (d, 1H, J = 5.2 Hz); 8.54 (d, 1H, J = 5.2 Hz); 8.89 (d, 1H, J = 5.2 Hz); 9.11 (d, 1H, J = 5.6 Hz). ^{13}C NMR ($CDCl_3$) 44.39 (2C); 114.03; 117.24; 119.27; 119.72; 123.95; 138.71; 146.66; 147.09; 148.74; 150.46; 151.14; 151.75; 156.77; 181.74. IR ($CHCl_3$) 1689 cm^{-1}. MS *m*/*z* 276 (24); 275 (32); 261 (100); 260 (95); 247 (34); 246 (38). t_R is 4.08 min (97.3% purity), using system I (CH_3CN/H_2O/TFA 10:90:0.1), and t_R is 14.34 min (98.8%purity), using system II (isooctane/EtOH 70:30), flow rate 2 mL/min.

4-Methoxy-7*H*-pyrido[4,3,2-*de*][1,10]phenanthrolin-7-one (9c). Method B was used and involved a mixture of compound **9a** (100 mg, 0.39 mmol) and DMF−DEA (0.27 mL, 1.58 mmol) in DMF (0.7 mL) which was warmed at 120 °C, under nitrogen atmosphere for 1 h. After concentration, NH_4Cl (0.6 g, 11.2 mmol) and ethanol (90 mL) were added, and the mixture was refluxed for 30 min. After concentration H_2O (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). After drying, the organic layers were concentrated, and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to give the expected tetracyclic compound as a brown solid (85 mg, 83%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 4.27 (s, 3H); 7.65 (dd, 1H, J = 4.8 and 8.0 Hz); 8.15 (d, 1H, J = 6.0 Hz); 8.70 (s, 1H); 8.78 (dd, 1H, J = 8.0 and 1.9 Hz); 9.10 (d, 1H, J = 6.0 Hz); 9.13 (dd, 1H, J = 1.9 and 4.8 Hz). ^{13}C NMR ($CDCl_3$) 56.97; 115.63; 120.81; 125.52; 129.02; 129.16; 130.22; 136.24; 139.81; 147.37; 149.31; 151.65; 153.07; 154.81; 180.34. IR ($HCCl_3$) 1674 cm^{-1}. t_R is 4.57 min (99.5% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 5.59 min (99.4%purity), using system II (isooctane/EtOH 70:30), flow rate 2 mL/min.

4-Methoxy-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (9d). Method B was used and involved a mixture of compound **9b** (100 mg, 0.39 mmol), DMF-DEA (0.27 mL, 1.58 mmol) in DMF (0.7 mL) which was warmed at 120 °C, under nitrogen atmosphere for 1 h. After concentration, NH_4Cl (0.6 g, 11.2 mmol) and ethanol (90 mL) were added, and the mixture was refluxed for 30 min. After concentration, H_2O (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). After drying, the organic layers were concentrated, and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to give the expected tetracyclic compound as a yellow solid (60 mg, 59%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 4.27 (s, 3H); 7.74 (dd, 1H, J = 4.4 and 8.1 Hz); 8.08 (d, 1H, J = 5.6 Hz); 8.72 (s, 1H); 8.93 (d, 1H, J = 5.6 Hz); 9.05 (dd, 1H, J = 1.9 and 4.4 Hz); 9.19 (dd, 1H, J = 1.9 and 8.1 Hz). ^{13}C NMR ($CDCl_3$) 57.03; 115.16; 119.70; 127.69; 129.48; 130.15; 132.86; 133.74; 140.82; 146.80; 147.98; 148.63; 152.81; 152.98; 179.84. IR ($HCCl_3$) 1679 cm^{-1}. t_R is 13.75 min (99.5% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 11.45 min (95.3%purity), using system II (isooctane/EtOH 80:20), flow rate 1 mL/min.

10-Ethoxycarbonyl-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (10d). Method C was used and involved a mixture of compound **10b′** (30 mg, 0.07 mmol) and TFA (0.27 mL, 3.5 mmol) which was stirred for 64 h. After evaporation of TFA, CH_2Cl_2 (15 mL) and $NaHCO_3$ (10 mL) were added. Concentration of the organic layer and purification of the crude product by flash chromatography (CH_2Cl_2) gave the expected tetracyclic compound as a yellow solid (11.3 mg, 53%), mp 246 °C. ^{1}H NMR ($CDCl_3$) 1.49 (t, 3H, J = 7.3 Hz); 4.53 (q, 2H, J = 7.3 Hz); 7,85 (d, 1H, J = 5.9 Hz); 8.03 (d, 1H, J = 5.5 Hz); 8.98 (d, 1H, J = 5.9 Hz); 9.22 (d, 1H, J = 5.5 Hz); 9.56 (d, 1H, J = 1.9 Hz); 9.73 (d, 1H, J = 1.9 Hz). ^{13}C NMR ($CDCl_3$) 14.32; 62.29; 119.61; 120.39; 124.04; 129.94; 132.60; 135.46; 138.77; 147.74; 148.78; 149.17; 149.46; 153.23; 164.15; 180.20 (1C not observed). IR ($CHCl_3$) 1694; 1726 cm^{-1}. MS: *m*/*z* 305 (91); 304 (100); 260 (7); 232 (93); 204 (24). t_R is 5.16 min (94.5% purity), using system I (CH_3CN/H_2O/TFA 50:50:0.1), and t_R is 9.13 min (98.6%purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

9-Hydroxy-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (11d). Method C was used and involved a mixture of compound **11b′** (50 mg, 0.135 mmol) and TFA (0.54 mL, 7 mmol) in CH_2Cl_2 (30 mL) which was stirred for 48 h. After evaporation of TFA, a saturated solution of $NaHCO_3$ (13 mL) was added, and the mixture was extracted with CH_2Cl_2 (7 × 30 mL). Concentration of the organic layer and purification of the crude product by flash chromatography (CH_2Cl_2/MeOH 97:2) gave the expected tetracyclic compound as an orange solid (16.8 mg, 50%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 7.17 (d, 1H, J = 8.8 Hz); 7.69 (d, 1H, J = 5.9 Hz); 7.93 (d, 1H, J = 5.5 Hz); 8.85 (d, 1H, J = 5.9 Hz); 8.99 (d, 1H, J = 8.8 Hz); 9.16 (d, 1H, J = 5.5 Hz). IR ($CHCl_3$) 1690; 1667; 1602 cm^{-1}. MS: *m*/*z* 249 (100); 221 (78); 193 (99). t_R is 11.41 min (99.7% purity), using system I (CH_3CN/H_2O/TFA 20:80:0.1), and t_R is 11.63 min (97.8% purity), using system II (isooctane/EtOH 80:20), flow rate 2 mL/min.

9-Methoxy-7*H*-pyrido[4,3,2-*de*][1,10]phenanthrolin-7-one (12c). Method B was used and involved a mixture of compound **12a** (83 mg, 0.33 mmol), DMF-DEA (0.21 mL, 1.23 mmol) in DMF (1 mL) which was warmed at 120 °C, under nitrogen atmosphere for 1 h. After concentration, NH_4Cl (0.48 g, 8.97 mmol) and ethanol (80 mL) were added, and the mixture was refluxed for 30 min. After concentration, a saturated solution of $NaHCO_3$ (30 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 60 mL). After drying, the organic layers were concentrated, and the crude product

was purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to give the tetracyclic compound as yellow solid (55 mg, 64%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 4.30 (s, 3H); 7.05 (d, 1H, J = 8.8 Hz); 7.83 (d, 1H, J = 5.5 Hz); 7.97 (d, 1H, J = 5.5 Hz); 8.64 (d, 1H, J = 8.8 Hz); 9.10 (d, 1H, J = 5.5 Hz); 9.16 (d, 1H, 5.5 Hz). ^{13}C NMR ($CDCl_3$) 54.64; 114.60; 120.50; 120.55; 123.65; 124.45; 138.57; 138.97; 147.38; 148.13; 148.53; 150.67; 151.51; 167.58; 180.88. IR ($CHCl_3$) 1669, 1593 cm^{-1}. MS: *m*/*z* 263 (77); 233 (99); 204 (35). t_R is 6.78 min (99.7% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 20.43 min (97.8%purity), using system II (isooctane/EtOH 80:20), flow rate 1 mL/min.

9-Methoxy-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (12d). Method B was used and involved a mixture of compound **12b** (200 mg, 0.79 mmol) and DMF−DEA (0.47 mL, 2.74 mmol) in DMF (3.2 mL) which was refluxed, under nitrogen atmosphere for 2 h. After concentration, NH_4Cl (1.4 g, 26.2 mmol) and ethanol (200 mL) were added, and the mixture was refluxed for 30 min. After concentration, H_2O (50 mL) was added, and the mixture was extracted with CH_2Cl_2 (5 × 40 mL). After drying, the organic layers were concentrated, and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH 99:1) to give the tetracyclic compound as a brown solid (20 mg, 10%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 4.14 (s, 3H); 7.11 (d, 1H, J = 8.8 Hz); 7.63 (d, 1H, J = 5.5 Hz); 7.87 (d, 1H, J = 5.5 Hz); 8.77 (d, 1H, J = 5.5 Hz); 8.91 (d, 1H, J = 8.8 Hz); 9.09 (d, 1H, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$) 53.41; 117.66; 118.54; 118.93; 123.70; 127.73; 136.29; 138.52; 145.95; 147.45; 148.03; 148.83; 150.19; 165.86; 180.55. IR ($CHCl_3$) 1686 cm^{-1}. MS: *m*/*z* 263 (8); 233 (25); 204 (30). t_R is 12.87 min (99.6% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 11.14 min (96.0% purity), using system II (isooctane/EtOH 80:20), flow rate 1 mL/min.

9,11-Dimethoxy-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (14d). Method B was used and involved a mixture of compound **14b** (105 mg, 0.37 mmol) and DMFDEA (0.22 mL, 1.29 mmol) in DMF (1.5 mL) which was warmed at 120 °C for 1.5 h. After concentration, NH_4Cl (0.7 g, 13.1 mmol) and EtOH (95 mL) were added, and the mixture was refluxed for 30 min. After concentration, H_2O (50 mL) was added and the mixture extracted with CH_2Cl_2 (5 × 40 mL). Flash chromatography (CH_2Cl_2/MeOH 99:1) gave the expected compound as an orange solid (7 mg, 9%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 4.12 (s, 3H); 4.18 (s, 3H); 6.65 (s, 1H); 7.64 (d, 1H, J = 5.5 Hz); 7.92 (d, 1H, J = 5.5 Hz); 8.93 (d, 1H, J = 5.5 Hz); 9.14 (d, 1H, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$) 54.39; 57.02; 98.26; 117.89; 118.64; 118.86; 124.16; 138.50; 146.93; 147.09; 148.29; 148.62; 151.50; 166.32; 167.73; 180.65. IR ($CHCl_3$) 1688 cm^{-1}. MS: *m*/*z* 293 (15); 292 (28); 233 (24); 204 (13); 165 (10). t_R is 3.75 min (99.7% purity), using system I (CH_3CN/H_2O/TFA 20:80:0.1), and t_R is 8.06 min (99.4%purity), using system II (isooctane/EtOH 70:30), flow rate 2 mL/min.

9-Chloro-11-(dimethylamino)-7*H*-pyrido[4,3,2-*de*][1,7]-phenanthrolin-7-one (15d). Method B was used and involved a mixture of compound **13b** (110 mg, 0.387 mmol) and DMFDEA (0.23 mL, 1.34 mmol) in DMF (1.1 mL) which was warmed at 120 °C for 1.5 h. After concentration, NH_4Cl (0.7 g, 13.1 mmol) and EtOH (95 mL) were added, and the mixture was refluxed for 30 min. After concentration, H_2O (50 mL) was added and the mixture extracted with CH_2Cl_2 (5 × 40 mL). Flash chromatography (CH_2Cl_2/MeOH 99:1) gave the expected compound as a red-purple solid (3.3 mg, 3%), mp 246 °C. ^{1}H NMR ($CDCl_3$) 3.04 (s, 6H); 7.11 (s, 1H); 7.61 (d, 1H, J = 5.5 Hz); 7.92 (d, 1H, J = 5.5 Hz); 8.90 (d, 1H, J = 5.5 Hz); 9.14 (d, 1H, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$) 44.39; 113.57; 117.60; 119.00; 119.37; 123.99; 138.50; 146.51; 146.77; 148.83; 150.68; 150.89; 153.68; 158.21; 180.05. IR ($CHCl_3$) 1698 cm^{-1}. MS: *m*/*z* 311 (19); 309 (11); 296 (89); 294 (100); 269 (4); 267 (1); 204 (66). t_R is 5.85 min (99.7% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 10.87 min (99.3% purity), using system II (isooctane/EtOH 70:30), flow rate 2 mL/min.

4,8-Dimethoxy-7*H*-pyrido[4,3,2-*de*][1,10]phenanthrolin-7-one (17c). Method B was used and involved a mixture of compound **17a** (330 mg, 1.17 mmol), DMFDEA (0.9 mL, 5.25 mmol) in DMF (3.5 mL) which was warmed at 120 °C for 4 h. After concentration, NH_4Cl (2.0 g, 37.4 mmol) and MeOH (300 mL) were added and the mixture was refluxed for 9 h. After concentration, H_2O (200 mL) was added and the mixture extracted with CH_2Cl_2 (4 × 200 mL). flash chromatography (CH_2Cl_2/MeOH 97:3) gave the expected compound as a brown solid (56 mg 17%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 4.13 (s, 3H); 4.24 (s, 3H); 7.13 (d, 1H, J = 5.9 Hz); 8.11 (d, 1H, J = 5.9 Hz); 8.67 (s, 1H); 8.93 (d, 1H, J = 5.9 Hz); 9.09 (d, 1H, J = 5.9 Hz). ^{13}C NMR ($CDCl_3$) 56.71; 56.88; 109.08; 115.72; 119,42; 120.02; 129.02; 130.19; 140.83; 147.54; 149.82; 152.48; 154.30; 155.30; 167.62; 179.75. IR ($CHCl_3$) 1670 cm^{-1}. MS: *m*/*z* 293 (44); 279 (13); 278 (100). t_R is 3.67 min (100% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 12.89 min (99.5% purity), using system II (isooctane/EtOH 70:30), flow rate 2 mL/min.

4,11-Dimethoxy-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (17d). Method B was used and involved a mixture of compound **17b** (100 mg, 0.35 mmol) and DMFDEA (0.24 mL, 1.23 mmol) in DMF (1 mL) which was warmed at 120 °C for 1.5 h. After concentration, NH_4Cl (0.6 g, 11.2 mmol) and EtOH (100 mL) were added, and the mixture was refluxed for 30 min. After concentration, H_2O (30 mL) was added and the mixture extracted with $CHCl_3$ (3 × 75 mL). Flash chromatography ($CHCl_3$/MeOH 95:5) gave the expected compound as a yellow solid (27 mg, 26%), mp > 260 °C. ^{1}H NMR (DMSO-d_6) 4.08 (s, 3H); 4.26 (s, 3H); 7.54 (d, 1H, J = 5.9 Hz); 7,98 (d, 1H, 5,9 Hz); 8,77 (d, 1H, J = 5.9 Hz); 8.83 (s, 1H); 8.94 (d, 1H, J = 5.9 Hz). ^{13}C NMR (DMSO-d_6) 57.41; 58.07; 112.43; 113.75; 119.84; 122.13; 129.60; 130.54; 140.17; 146.81; 150.17; 150.62; 153.03; 153.35; 166.06; 179.30. IR ($CHCl_3$) 1682; 1608; 1572 cm^{-1}. MS: *m*/*z* 293 (34); 292 (42); 220 (19); 192 (30); 165 (22). t_R is 3.71 min (98.0% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 16.60 min (95.3%purity), using system II (isooctane/EtOH 70:30), flow rate 2 mL/min.

4-Methoxy-11-(dimethylamino)-7*H*-pyrido[4,3,2-*de*][1,7]-phenanthrolin-7-one (18d). Method B was used and involved a mixture of compound **18b** (80 mg, 0.27 mmol) and DMFDEA (0.18 mL, 1.05 mmol) in DMF (2 mL) which was warmed at 120 °C for 3 h. After concentration, NH_4Cl (0.4 g, 7.48 mmol) and EtOH (90 mL) were added, and the mixture was refluxed for 30 min. After concentration, H_2O (30 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 50 mL). flash chromatography (CH_2Cl_2/MeOH 95:5) gave the expected compound as a red solid (33 mg, 40%), which decomposes before melting. ^{1}H NMR ($CDCl_3$) 3.02 (s, 6H); 4.23 (s, 3H); 7.08 (d, 1H, J = 5.9 Hz); 7.87 (d, 1H, J = 5.5 Hz); 8.54 (d, 1H, J = 5.9 Hz); 8.65 (s, 1H); 8.90 (d, 1H, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$) 44.28; 56.94; 112.14; 113.63; 119.38; 119.73; 129.31; 129.99; 140.20; 145.81; 150.31; 150.63; 151.41; 152.99; 156.77; 180.57. IR ($CHCl_3$) 1682 cm^{-1}. MS: *m*/*z* 306 (52); 305 (32); 291 (100); 290 (66); 276 (24); 248 (9); 220 (13); 193 (21). t_R is 3.69 min (100% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 10.51 min (100%purity), using system II (isooctane/EtOH 70:30), flow rate 2 mL/min.

4,9-Dimethoxy-7*H*-pyrido[4,3,2-*de*][1,7]phenanthrolin-7-one (19d). Method B was used and involved a mixture of compound **19b** (100 mg, 0.35 mmol) and DMFDEA (0.24 mL, 1.4 mmol) in DMF (1 mL) which was warmed at 120 °C for 1 h. After concentration, NH_4Cl (0.54 g, 10.1 mmol) and EtOH (100 mL) were added, and the mixture was refluxed for 30 min. After concentration, H_2O (20 mL) was added and the mixture extracted with $CHCl_3$ (3 × 30 mL). Flash chromatography ($CHCl_3$) gave the expected compound as a green solid (37 mg, 36%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 4.21 (s, 3H); 4.24 (s, 3H); 7.16 (d, 1H, J = 8.8 Hz); 7.98 (d, 1H, 5.6 Hz); 8.69 (s, 1H); 8.85 (d, 1H, J = 5.6 Hz); 9.00 (d, 1H, J = 8.8 Hz). ^{13}C NMR (DMSO-d_6) 54.44; 56.92; 114.04; 117.17; 118.86; 127.74; 129.43; 129.99; 136.29; 141.16; 146.36; 146.72; 149.38; 152.94; 165.80; 179.70. IR ($CHCl_3$) 1679 cm^{-1}. MS: *m*/*z* 293 (44); 248 (100); 220 (12). t_R is 5.82 min (95.1% purity), using system I (CH_3CN/H_2O/TFA 30:70:0.1), and t_R is 3.31 min (98.0%purity), using system II (isooctane/EtOH 70:30), flow rate 2 mL/min.

Pharmacology. In Vitro Characterization of Drug-Induced Effects with Respect to 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 (A-172, U-373 MG and U-87 MG), two colon (HCT-15 and LoVo), two non-small-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 CRL1620 (A-172), 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 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 40000 cells /mL culture medium) to ensure adequate plating prior to cell growth determination, which was carried out by means of the colorimetric MTT assay, as detailed previously.[11,12] This assessment of cell population growth is based on the capability of living cells to reduce the yellow product MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 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 compounds under study (see Table 1).

In Vivo Determination of Drug-Induced Toxicity. Drug-induced toxicity can be monitored in vivo by determining the maximum tolerated dose (MTD). This MTD determination is carried out by defining the maximum dose of the drug which 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 χ^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 the statistical analyses were carried out using Statistica (Statsoft, Tulsa, OK).

References

(1) Schmitz, F. J.; Agarwal, S. K.; Gunasekera, S. P. Amphimedine, new aromatic alkaloid from a pacific sponge, *Amphimedon* sp. Carbon connectivity determination from natural abundance $^{13}C-^{13}C$ coupling constants. *J. Am. Chem. Soc.* **1983**, *105*, 4835−4836.

(2) Nilar; Sidebottom, P. J.; Carté, B. K.; Butler, M. S. Three new pyridoacridine type alkaloids from a singaporean ascidian. *J. Nat. Prod.* **2002**, *65*, 1198−1200.

(3) (a) Molinski, T. F. Marine pyridoacridine alkaloids: structure, synthesis, and biological chemistry. *Chem. Rev.* **1993**, *93*, 1825−1838. (b) Ding, Q.; Chichak, K.; Lown, J. W. *Curr. Med. Chem.* **1999**, *6*, 1−27. (c) Delfourne, E.; Bastide, J. Marine pyridoacridine alkaloids and synthetic analogues as antitumor agents. *Med. Res. Rev.* **2003**, *23*, 234−252.

(4) (a) Delfourne, E.; Darro, F.; Bontemps-Subielos, N.; Decaestecker, C.; Bastide, J.; Frydman, A.; Kiss, R. Synthesis and characterization of the antitumor activities of analogues of meridine, a marine pyridoacridine alkaloid. *J. Med. Chem.* **2001**, *44*, 3275−3282. (b) Delfourne, E.; Darro, F.; Portefaix, P.; Galaup, C.; Bayssade, S.; Bouteille, A.; Le Corre, L.; Bastide, J.; Collignon, F.; Lesur, F.; Frydman, A.; Kiss, R. Synthesis and in vitro antitumor activity of novel ring D analogues of the marine pyridoacridine ascididemin: structure−activity relationship. *J. Med. Chem.* **2002**, *45*, 3765−3771. (c) Brahic, C.; Darro, F.; Belloir, M.; Bastide, J.; Kiss, R.; Delfourne, E. Synthesis and cytotoxic evaluation of analogues of the marine pyridoacridine amphimedine. *Bioorg. Med. Chem.* **2002**, *10*, 2845−2853.

(5) Lindsay, B. S.; Barrows, L. R.; Copp, B. R. Structural requirement for biological activity of the marine alkaloid ascididemin. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 739−742.

(6) Matsumoto, S. S.; Sidford, M. H.; Holdel, J. A.; Barrows, L. R.; Copp, B. R. Mechanism of action studies of cytotoxic marine alkaloids: ascididemin exhibits thiol-dependent oxidative DNA cleavage. *Tetrahedron Lett.* **2000**, *41*, 1667−1670.

(7) Bracher, F. Total synthesis of the pentacyclic alkaloid ascididemin. *Heterocycles* **1989**, *29*, 2093−2095.

(8) Weinstein, J. N.; Kohn, K. W.; Grever, M. R.; Viswanadhan, V. N.; Rubinstein, L. V.; Monks, A. P.; Scudiero, D. A.; Welch, L.; Koutsoukos, A. D.; Chiausa, A. J.; Paull, K. D. Neural computing in cancer drug development: predicting mechanism of action. *Science* **1992**, *258*, 447−449.

(9) Kaslow, C. E.; Sommer, N. B. Substituted lepidines. *J. Am. Chem. Soc.* **1946**, 644−647.

(10) Kitahara, Y.; Onikura, H.; Shibano, Y.; Watanabe, S.; Mikami, Y.; Kubo, A. Synthesis of eupomatidines 1, 2 and 3 and related compounds including iminoquinolinequinone structure. *Tetrahedron* **1997**, *53*, 6001−6010.

(11) Pauwels, O.; Kiss, R.; Pasteels, J. L.; Atassi, G. Characterization of alkylating versus intercalating anticancer drug-induced effects on cell survival, cell cycle kinetic and morphonuclear pattern of three neoplastic cell lines growing in vitro. *Pharm. Res.* **1995**, *12*, 1011−1018.

(12) Camby, I.; Salmon, I.; Danguy, A.; Pasteels, J. L.; Brotchi, J.; Martinez, J.; Kiss, R. Influence of gastrin on human astrocytic tumor cell proliferation. *J. Natl. Cancer Inst.* **1996**, *88*, 594−600.

JM0308702