Synthesis and Characterization of the Antitumor Activities of Analogues of Meridine, a Marine Pyridoacridine Alkaloid

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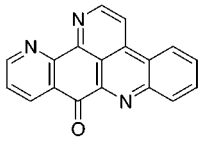
Marine compounds with pyridoacridine skeletons are known to exhibit interesting antitumor activities. Among these compounds, meridine has already been reported as having significant antitumor activities in vitro. We synthesized 24 analogues of meridine substituted on ring A with the aim of obtaining compounds that display significantly higher in vitro antitumor activities than meridine. The 24 compounds and meridine used as a control compound were tested at 6 different concentrations on 12 different human cancer cell lines including 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 25 compounds ranged over 5 log concentrations, i.e., between 10 and 0.0001 μM, with four of the compounds exhibiting a significantly higher in vitro antitumor activity than meridine. These compounds will now be subjected to further pharmacological investigation including in vivo testing on both conventional murine tumors and human tumors grafted onto nude mice.

Introduction

In the search for new anticancer drugs, metabolites from marine organisms (sponges, tunicates, bryozoa, and algae) have attracted considerable interest in the past 15 years because these organisms contain cytotoxic compounds with unique molecular structures.[1] The largest family of marine alkaloids characterized to date is based on the pyrido[*k*,*l*]acridine skeleton.[2] These polycyclic aromatic compounds have been reported as exhibiting very interesting biological properties, including the inhibition of a variety of cultured cell clones at micromolar concentrations.[3] From the few published studies on the mechanism of action of these structures, two general properties of pyridoacridine alkaloids have emerged so far: (a) They are DNA intercalating agents, and (b) nucleic acid intercalation is further modulated by binding to other receptors (topoisomerases, transition metals, etc.).[4] Recently, other original mechanisms have been reported that involve cytotoxicity action. Matsumoto et al.[5] have shown that ascididemin **1** exhibits a thiol-dependent oxidative DNA cleavage. De Guzman et al.[6] have shown that neoamphimedine **2** has the novel ability to stimulate topoisomerase II to catenate DNA to a high molecular weight complex. From all these results, it appears that pyridoacridine derivatives are good candidates for the discovery of compounds with unique mechanisms and selectivity associated with antitumor activity. Among these, we are especially interested in meridine **3**, which was first isolated by Schmitz et al.[7] from the ascidian *Amphicarpa meridiana*, and which has been described as having antifungal[8] and antitumoral[3] properties. To our knowledge, only two papers have been published to date on the cytotoxic characteristics of meridine.[3,8]



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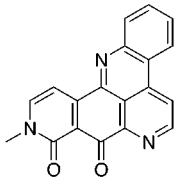

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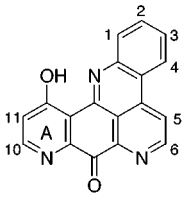

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We set out to prepare a series of analogues of **3** substituted on ring A with a view to investigating in vitro the effect of the substituents on antitumor activity. The synthesis and assessment of the in vitro antitumor activity of these substances are described in the present report.

Chemistry

The different meridine-like structures were synthesized on the basis of Kubo's strategy for the synthesis of meridine.[9] The hetero-Diels−Alder-reactions were carried out between different quinoline-5,8-dione derivatives and *o*-nitro- or o-trifluoroacetamidocinnamaldehyde-dimethylhydrazone (Scheme 1).

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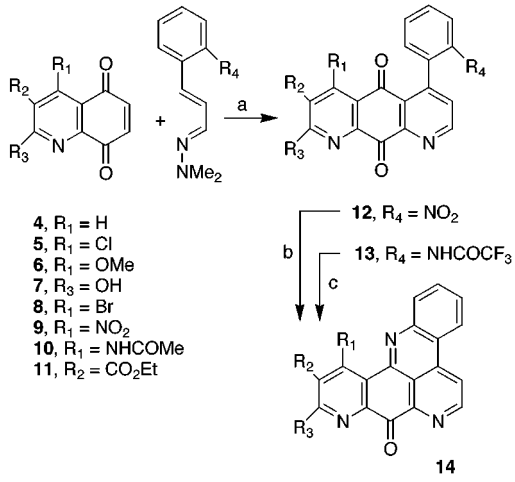
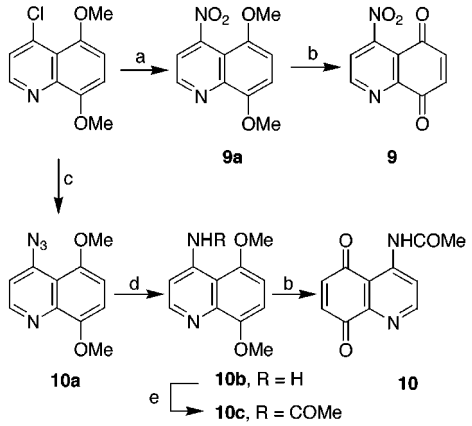
Scheme 1[a]



[a] (a) CH_3CN, Ac_2O, reflux; 10% Pd/C, toluene, reflux. (b) H_2, 10% Pd/C. (c) CF_3COOH or NaOH, CH_2Cl_2, reflux.

Scheme 2[a]



[a] (a) $NaNO_2$, tetrabutylammoniun chloride, CH_2Cl_2/H_2O, room temp, 3 days. (b) CAN, CH_3CN/H_2O, room temp, 15 min. (c) NaN_3, DMF/H_2O, 90 °C, 2h and 30 min. (d) Ph_3P, THF/H_2O, room temp, 2 h. (e) Ac_2O, DMAP, room temp, 24 h.

Quinoline-5,8-dione[10] (**4**), 4-chloro, 4-methoxyquinoline-5,8-dione (respectively **5**[11] and **6**[12]), and 5,8-dioxocarbostyril (**7**[13]) were obtained according to procedures previously described. 4-Bromoquinoline-5,8-dione (**8**) was prepared in two steps from 5,8-dimethoxyquinolin-4-ol triflate, i.e., bromination and the subsequent oxidation by cerium ammonium nitrate. The addition of sodium nitrite to 4-chloro-5,8-dimethoxyquinoline and oxidation by cerium ammonium nitrate led to **9**. 4-Chloro-5,8-dimethoxyquinoline was first transformed by the action of sodium azide into the azido compound **10a**, which was then reduced to amine **10b** by triphenylphosphine; this amine was acetylated by acetic anhydride, leading to the acetamido derivative **10c**, which was oxidized by cerium ammonium nitrate in compound **10** (Scheme 2).

3-Ethyl-5,8-dimethoxyquinolinecarboxylate[14] was also oxidized by cerium ammonium nitrate to give the corresponding quinolinedione **11**.

The nitro derivatives **12a**,[15] **12b**,[9] and **12c**[15] were synthesized according to methods previously described. **12j** was synthesized by a similar Diels−Alder reaction. **12i** was obtained by the same procedure as **12c**, i.e., the treatment of **12a** by sodium azide in DMF but in the absence of water. Compound **13c** was prepared from **13a** by the action of sodium azide in DMF, with the reduction of the azido intermediate in amine occurring spontaneously in the reaction medium as described by Kitahara et al.[15] in the case of **12c**. The *o*-trifluoroacetamido adducts **13a**, **13b**, **13d**−**f**, **13h**, **13j**, and **13k** were also obtained by a Diels−Alder addition, which proceeded regioselectively but with low yields. The **13g** derivative was prepared from chloro analogue **13a** by the action of dimethylamine. The aromatization of the Diels−Alder adducts was realized by the treatment of the crude product with 10% Pd/C in toluene. The catalytic hydrogenation of derivatives **12a**−**c**,**i**,**j** or the acidic or alkaline hydrolysis of the trifluoroacetamido group of compounds **13a**−**g**,**j**,**k** gave the amino compounds, which cyclized in situ to yield pentacyclic pyridoacridines **14a**−**g**,**j**,**k**. Compound **13h** was the only one that could not be transformed into its pentacyclic parent. The different compounds obtained are reported in Table 1.

Table 1

R_1	R_2	R_3	nitro derivatives **12**	trifluro-acetamido derivatives **13**	pentacyclic derivatives **14**
Cl	H	H	**12a**	**13a**	**14a**
OMe	H	H	**12b**	**13b**	**14b**
NH_2	H	H	**12c**	**13c**	**14c**
Br	H	H		**13d**	**14d**
H	H	H		**13e**	**14e**
NO_2	H	H		**13f**	**14f**
NMe_2	H	H		**13g**	**14g**
NHCOMe	H	H		**13h**	
OH	H	H	**12i**		**3**
H	CO_2Et	H	**12j**	**13j**	**14j**
H	H	OH		**13k**	**14k**

Pharmacology: In Vitro Determination of the Drug-Induced Inhibition of Human Cancer Cell Line Growth

Six concentrations of each of the 25 compounds under study were tested on 12 different human cancer cell lines including various histopathological types (glioblastomas and breast, colon, lung, prostate, and bladder cancers). This experimental approach enabled us to determine the IC_{50} value for each drug, i.e., the concentration that reduced the mean growth value of the 12 cell lines by 50% compared to the mean control growth value. Table 2 illustrates the individual IC_{50} values obtained for each of the 12 cell lines under study. These data show that IC_{50} values ranged between 10 and 0.0001 μM. They thus ranged over 5 logarithmic concentrations. The compounds that exhibited the highest in vitro antitumor activities included **13b**, **13e**, **14b**, and **14e**. These newly synthesized compounds therefore appeared markedly more efficient in vitro in terms of antitumoral activity than the parent drug from which they were derived, i.e., meridine (Table 2). Indeed, the meridine-associated IC_{50} values ranged between 10 and 0.01 μM over the 12 cell lines under study (Table 2). The 12 cell lines exhibited differences in terms of drug sensitivity, which varied over more than 3 logarithmic concentrations for a given compound. This is the case for compound **13e**, for example.

Table 2. Characterization of the in Vitro Cytotoxic-Related Anti-tumor Effects (IC50 Value in μM) of the Compounds Listed in Table 1[a]

	cell line											
compd	U-87MG	U-373MG	SW1088	T24	J82	HCT-15	LoVo	MCF7	T-47D	A549	A-427	PC-3
12a	2 ± 0.1	4.8 ± 0.5	5.4 ± 0.2	5.5 ± 0.4	0.5 ± 0.05	2.8 ± 0.2	3.9 ± 0.3	1.8 ± 0.1	0.8 ± 0.06	0.9 ± 0.06	3.5 ± 0.3	5 ± 0.6
12b	0.009 ± 0.0004	0.03 ± 0.005	3.9 ± 0.2	0.02 ± 0.001	0.4 ± 0.03	0.09 ± 0.005	0.05 ± 0.008	0.05 ± 0.01	5.1 ± 0.3	0.02 ± 0.002	0.7 ± 0.05	3.1 ± 0.4
12c	2.7 ± 0.2	3.1 ± 0.6	4.8 ± 0.2	3.6 ± 0.2	3 ± 0.6	0.5 ± 0.03	5.7 ± 0.3	3.1 ± 0.3	5.5 ± 0.5	0.6 ± 0.07	5.5 ± 0.7	5.1 ± 0.4
12i	0.8 ± 0.03	4 ± 0.5	2.9 ± 0.08	2.3 ± 0.2	5.3 ± 0.7	3.5 ± 0.7	2.9 ± 0.4	3.9 ± 0.2	4.5 ± 0.3	2.3 ± 0.2	1.2 ± 0.2	7.3 ± 0.7
12j	not available											
13a	0.05 ± 0.002	0.09 ± 0.003	3.8 ± 0.3	0.2 ± 0.01	3.3 ± 0.5	0.01 ± 0.0009	0.06 ± 0.004	0.06 ± 0.005	2.1 ± 0.2	0.07 ± 0.008	0.6 ± 0.07	0.9 ± 0.08
13b	0.0001>	0.0001>	0.05 ± 0.005	0.007 ± 0.001	0.0009 ± 0.00006	0.002 ± 0.0001	0.0001 ± 0.00002	0.0003 ± 0.00005	0.03 ± 0.006	0.0001>	0.05 ± 0.007	0.05 ± 0.01
13c	0.3 ± 0.02	0.9 ± 0.02	0.8 ± 0.05	0.7 ± 0.04	2.7 ± 0.2	0.6 ± 0.06	0.7 ± 0.05	0.8 ± 0.1	1.2 ± 0.1	0.6 ± 0.1	0.5 ± 0.05	0.6 ± 0.1
13d	not available											
13e	0.0009 ± 0.00004	0.005 ± 0.0005	0.06 ± 0.003	0.2 ± 0.01	0.06 ± 0.004	0.008 ± 0.0004	0.007 ± 0.0006	0.006 ± 0.0006	0.05 ± 0.008	0.006 ± 0.0005	0.03 ± 0.002	0.06 ± 0.006
13f	4.7 ± 0.2	0.9 ± 0.04	6 ± 0.3	5.4 ± 0.4	3.9 ± 0.2	0.6 ± 0.08	0.9 ± 0.08	0.7 ± 0.08	4.3 ± 0.3	0.9 ± 0.06	4.5 ± 0.5	0.6 ± 0.03
13g	0.06 ± 0.003	0.3 ± 0.02	0.4 ± 0.04	0.3 ± 0.01	2 ± 0.1	0.2 ± 0.02	0.1 ± 0.008	0.1 ± 0.04	0.7 ± 0.06	0.06 ± 0.004	0.07 ± 0.005	0.6 ± 0.03
13h	0.06 ± 0.001	0.09 ± 0.006	0.09 ± 0.006	0.4 ± 0.03	3.5 ± 0.2	0.09 ± 0.006	0.07 ± 0.006	0.09 ± 0.01	0.3 ± 0.02	0.07 ± 0.005	0.06 ± 0.006	2.7 ± 0.2
13j	0.08 ± 0.002	0.4 ± 0.02	0.8 ± 0.006	0.6 ± 0.1	5.2 ± 0.3	0.1 ± 0.007	0.8 ± 0.07	0.5 ± 0.03	2.6 ± 0.2	0.09 ± 0.009	0.6 ± 0.05	1.3 ± 0.1
13k	0.04 ± 0.001	0.04 ± 0.003	0.06 ± 0.003	0.05 ± 0.005	2.1 ± 0.1	0.06 ± 0.007	0.04 ± 0.005	0.03 ± 0.003	0.2 ± 0.02	0.05 ± 0.008	0.04 ± 0.003	0.9 ± 0.02
14a	0.02 ± 0.004	0.1 ± 0.02	4.3 ± 0.2	0.09 ± 0.006	0.09 ± 0.005	0.1 ± 0.005	0.05 ± 0.006	0.08 ± 0.005	2.7 ± 0.2	0.007 ± 0.001	0.9 ± 0.06	0.5 ± 0.1
14b	0.0001>	0.0001>	0.06 ± 0.004	0.006 ± 0.001	0.0009 ± 0.0001	0.002 ± 0.0004	0.0003 ± 0.00002	0.0001>	0.03 ± 0.002	0.0003 ± 0.00003	0.005 ± 0.0006	0.06 ± 0.008
14c	0.07 ± 0.003	0.1 ± 0.03	0.08 ± 0.004	0.07 ± 0.005	0.7 ± 0.05	0.3 ± 0.06	0.09 ± 0.006	0.09 ± 0.006	0.6 ± 0.05	0.08 ± 0.007	0.06 ± 0.008	0.5 ± 0.06
14d	0.7 ± 0.03	0.9 ± 0.02	3 ± 0.3	0.7 ± 0.07	9 ± 0.5	3.6 ± 0.4	0.8 ± 0.09	1.1 ± 0.06	4.4 ± 0.3	3.3 ± 0.6	0.7 ± 0.06	4.9 ± 0.3
14e	0.0001>	0.0007 ± 0.00006	0.06 ± 0.01	0.0001>	0.007 ± 0.0004	0.005 ± 0.0004	0.02 ± 0.002	0.0009 ± 0.00008	0.01 ± 0.001	0.02 ± 0.004	0.0008 ± 0.0001	0.04 ± 0.004
14f	0.07 ± 0.004	0.09 ± 0.004	5.5 ± 0.8	0.7 ± 0.05	0.5 ± 0.03	0.6 ± 0.04	0.09 ± 0.006	0.07 ± 0.004	0.6 ± 0.2	0.8 ± 0.08	2.4 ± 0.3	4.2 ± 0.4
14g	0.05 ± 0.004	0.08 ± 0.004	0.1 ± 0.01	0.2 ± 0.02	1.3 ± 0.08	0.07 ± 0.006	0.08 ± 0.01	0.08 ± 0.006	0.5 ± 0.1	0.06 ± 0.01	0.06 ± 0.003	0.6 ± 0.04
3	0.1 ± 0.02	0.1 ± 0.07	2 ± 0.1	0.05 ± 0.004	8.2 ± 0.5	0.2 ± 0.02	0.07 ± 0.01	0.09 ± 0.009	0.07 ± 0.008	0.08 ± 0.02	0.2 ± 0.03	0.5 ± 0.05
14j	0.07 ± 0.002	0.7 ± 0.05	2.8 ± 0.2	0.9 ± 0.1	5.2 ± 0.3	0.2 ± 0.02	0.7 ± 0.03	0.7 ± 0.05	2.9 ± 0.2	0.08 ± 0.02	0.8 ± 0.1	5.7 ± 0.3
14k	not available											

[a] The IC_{50} value constitutes the concentration of the compound that inhibits the growth of the human cancer cells by 50% compared to the control value. Results are reported as the IC_{50} mean ± standard error of the IC_{50} mean. 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 the cell line growth level were determined by means of the MTT colorimetric assay.

Discussion

Longley et al.[3] studied 24 metabolites (including pyridoacridine structures and others) from marine sponges and evaluated them for their cytotoxicity against two human tumor cell lines, i.e., the A549 nonsmall cell lung carcinoma (also used in our present study) and the HT-29 colon adenocarcinoma, and also against one murine leukemia cell line. Of these 24 compounds, meridine had a weaker level of antitumor activity than other compounds, including latrunculin A, batzelline A, and chondrillin.[3] We synthesized new meridine analogues and investigated whether any of them displayed any greater antitumor activity than the parent meridine. In the present work we were exclusively interested in substitutions on ring A, with R_1 being the most modulated position. The same strategy based on the Diels−Alder reaction and involving diverse substituted quinoline-5,8-diones enabled the different meridine derivatives to be synthesized. The yield (low in all cases) and the regioselectivity of the Diels−Alder reaction was not altered by the nature of the dienophile substituent. It should be noted that the aromatic dienes led to a unique regioisomer unlike the same reaction with aliphatic dienes,[16] in which the formation of two isomers was generally observed.

We made use of the colorimetric MTT assay, which indirectly assesses the effect of potentially anticancer compounds on the overall growth of adherent cell lines.[17] The data that we obtained in vitro showed that almost all the compounds have cytotoxic properties and that some of them had levels of antitumor activity up to 10 000 times higher than the parent meridine (see Table 2). These results could be in accordance with the mechanism proposed by Matsumoto et al. for another pyridoacridine: ascididemine.[5] These authors have demonstrated that this alkaloid can act to oxidatively damage DNA, probably via its iminoquinone moiety. Compounds **13** and **14** respectively possess a quinone or an iminoquinone function that could involve a redox reaction. The substituent effect is rather the same from one structure (**13**) to another (**14**), with the most active compounds being the unsubstituted ones and those with a methoxyl group in R_1.

To explain the results of the R_1 position of pentacyclic compounds, we investigated a number of PM3[18]-derived parameters such as the geometry of minimum energy and charge parameters. The most active compounds were those with two concomitant properties, i.e., first, PM3-optimized conformations close to planar structures, which is the case for compounds **14a**−**c** and **14j** and, second, the N(13) charge above −0.06; compounds **3** and **14c** exhibit a strong hydrogen bond between the hydrogen of the substituent and the N(13), leading to a high negative charge on N(13). The in vitro activity of these two compounds was lower than that of **14b** and **14e**.

Another hypothesis from the point of view of the mode of action is that the antitumor activity observed could relate, at least partly, to the inhibition of topoisomerase-II activity. Indeed, McDonald et al.[4] characterized the antitumor activities of several newly isolated pyridoacridine alkaloids (including dehydrokuanoniamine B, shermilamine C, cystodytin K, etc.) from a Fijian *Cystodytes sp*. ascidian. They pointed out that the pyridoacridines' ability to inhibit the topoisomerase-II-mediated decatenation of kinetoplast DNA correlates with their cytotoxic potency against human colon cancer cells. These authors[4] thus concluded that the disruption of the function of topoisomerase-II after DNA intercalation is a probable mechanism by which pyridoacridines inhibit the proliferation of human colon tumor cells.

In conclusion, we have now succeeded in synthesizing meridine analogues. Among these compounds **13b**, **14b**, **13e**, and **14e** have levels of in vitro cytotoxic antitumor activity up to 10 000 times greater than the parent meridine when directed against a panel of 12 human cancer cell lines. Whether the antitumor activities of the original meridine analogues that we synthesized are exerted through anti-topoisomerase action or through other mechanisms is not yet known. 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

1. Chemistry. Chemical Synthesis. ^{1}H 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) by means of the solvent systems indicated below. The purity of the different meridine analogues was evaluated on two analytical chromatographic systems. System I consisted of an ultrabase UB 235, 5 μm column (250 mm × 4.6 mm), H_2O/CH_3CN 50:50 at 1 mL/min flow rate, 200 nm, and the system II consisted of a Luna phenylhexyl, 5 μm column (150 mm × 4.6 mm), H_2O/CH_3CN 60:40 at 1.5 mL/min flow rate, 200 nm.

4-Bromo-5,8-dimethoxyquinoline. A mixture of 5,8-dimethoxyquinolin-4-ol triflate[9] (3 g, 9.35 mmol) and LiBr (8.2 g, 94.2 mmol) in dioxane (60 mL) was refluxed for 30 min. The mixture was cooled and H_2O (200 mL) added. The mixture was extracted with AcOEt (3 × 200 mL), and the extract was dried over $MgSO_4$ and concentrated to dryness to give the bromo derivative (3 g, 91%) as a yellow solid: mp, 86 °C. ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 3H), 4.02 (s, 3H), 6.89 (d, 1H, J = 8.8 Hz), 6.97 (d, 1H, J = 8.8 Hz), 7.71 (d, 1H, J = 4.4 Hz), 8.57(d,1H, J = 4.4 Hz). ^{13}C NMR ($CDCl_3$): δ 56.10, 56.26, 107.54, 107.89, 120.49, 128.08, 128.79, 141.81, 148.18, 148.85, 149.44.

4-Bromoquinoline-5,8-dione (8). Cerium ammonium nitrate (CAN) (0.6 g, 1.11 mmol) was added to a solution of 4-bromo-5,8-dimethoxyquinoline (0.1 g, 0,373 mmol), CH_3CN (8 mL), and H_2O (4 mL). The mixture was stirred at room temperature for 30 min. The CH_3CN was evaporated, and H_2O (100 mL) was added to the residue. The mixture was extracted with $HCCl_3$ (3 × 100 mL). The extract was dried over $MgSO_4$, and the solvent was evaporated to yield dione (83 mg, 93%) as a pink solid: mp, 190 °C. ^{1}H NMR ($CDCl_3$): δ 7.06 (d, 1H, J = 10 Hz), 7.12 (d, 1H, J = 10 Hz), 7.93 (d, 1H, J = 5.2 Hz), 8.73 (d, 1H, J = 5.2). ^{13}C NMR ($CDCl_3$): δ 126.71, 132.83, 134.13, 136.89, 139.16, 148.95, 152.88, 181.53, 182.6.

4-Nitro-5,8-dimethoxyquinoline (9a). A solution of 4-chloro-5,8-dimethoxyquinoline[11] (1.89 g, 8.47 mmol), $NaNO_2$ (0.69 g, 10.06 mmol), and tetrabutylammonium chloride, (2.9 g, 10.59 mmol) in CH_2Cl_2 (50 mL) and H_2O (50 mL) was stirred at room temperature for 3 days. The organic layer was recovered, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to dryness. Purification of the crude product by flash chromatography (CH_2Cl_2/MeOH, 98:2) gave the nitro compound (1.2 g, 60%) as a bright-yellow solid: mp, 169 °C. ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 3H), 4.05 (s, 3H), 6.92 (d, 1H, J = 8.8 Hz), 7.07 (d, 1H, J = 8.8 Hz), 7.36 (d, 1H, J = 4.4

Hz), 9.01 (d, 1H, $J = 4.4$ Hz). ^{13}C NMR ($CDCl_3$): δ 56.41, 56.52, 107.61, 108.99, 111.02, 113.69, 141.99, 146.48, 149.31, 149.53, 152.68.

4-Nitroquinoline-5,8-dione (9). 9 was prepared as described for **8**, from 4-nitro-5,8-dimethoxyquinoline (0.4 g, 1.71 mmol), CAN (2.8 g, 5.2 mmol), CH_3CN (10 mL), and H_2O (5 mL). After the mixture was stirred for 15 min, an orange solid was obtained (290 mg, 83%): mp, 180 °C. ^{1}H NMR ($CDCl_3$): δ 7.10 (d, 1H, $J = 10.4$ Hz), 7.23 (d, 1H, $J = 10.4$ Hz), 7.64 (d, 1H, $J = 5.6$ Hz), 9.24 (d, 1H, $J = 5.6$ Hz). ^{13}C NMR (DMSO-d_6): δ 118.49, 120.00, 137.89, 139.26, 148.83, 152.83, 156.38, 181.07, 182.04.

4-Azido-5,8-dimethoxyquinoline (10a). A solution of 4-chloro-5,8-dimethoxyquinoline (10 g, 44.7 mmol) and NaN_3 (16.7 g, mmoles) in a mixture DMF/H_2O (160 mL/60 mL) was heated at 90 °C for 2 h and 30 min. After the solution was cooled, a saturated solution of NH_4Cl (500 mL) was added and the mixture extracted by $CHCl_3$ (3 × 150 mL). The extract was dried over $MgSO_4$ and concentrated to dryness to give the azido compound (8.7 g, 85%) as a brown solid: mp, 106 °C. ^{1}H NMR ($CDCl_3$): δ 3.86 (s, 3H), 3.95 (s, 3H), 6.75 (d, 1H, $J = 8.6$ Hz), 6.89 (d, 1H, $J = 8.6$ Hz), 7.10 (d, 1H, $J = 4.8$ Hz), 8.72 (d, 1H, $J = 4.8$ Hz). ^{13}C NMR ($CDCl_3$): δ 55.77, 56.50, 106.44, 107.49, 110.76, 114.20, 142.25, 145.45, 148.82, 148.96, 149.13.

4-Amino-5,8-dimethoxyquinoline (10b). A solution of 4-azido-5,8-dimethoxyquinoline (8.7 g, 37.8 mmol) and triphenylphosphine (12 g, 45.8 mmol) was stirred at room temperature for 2 h in a THF/H_2O mixture (110 mL/110 mL). After concentration, 1 N HCl (200 mL) was added and the mixture extracted with ether (3 × 500 mL). The aqueous layer was then made alkaline by 1 N NaOH (250 mL) and extracted by $CHCl_3$ (3 × 250 mL). The extract was dried over $MgSO_4$ and concentrated to dryness to give quantitatively the amino derivative as a brown solid (which decomposes before melting). ^{1}H NMR ($CDCl_3$): δ 3.86 (s, 3H), 3.94 (s, 3H), 5.93 (br.s, 2H), 6.39 (d, 1H, $J = 5.2$ Hz), 6.55 (d, 1H, $J = 8.8$ Hz), 7.77 (d, 1H, $J = 8.8$ Hz), 8.36 (d, 1H, $J = 5.2$ Hz). ^{13}C NMR ($CDCl_3$): δ 55.77, 55.80, 102.22, 104.73, 106.32, 111.14, 142.45, 149.48, 149.55, 150.81, 152.09.

4-Acetamido-5,8-dimethoxyquinoline (10c). A suspension of 4-amino-5,8-dimethoxyquinoline (3 g, 14.7 mmol) and DMAP (0.72 g, 5.88 mmol) was stirred at room temperature for 24 h in acetic anhydride (30 mL). A saturated solution of $NaHCO_3$ (40 mL) was added after concentration, and the mixture was extracted with $CHCl_3$ (3 × 50 mL). The extract was dried over $MgSO_4$ and concentrated (benzene was added and the mixture concentrated to remove the acetic anhydride) to give the acetamido derivative (2.7 g, 74%) as a yellow solid: mp, 152 °C. ^{1}H NMR ($CDCl_3$): δ 2.26 (s, 3H), 4.03 (s, 6H), 6.81 (d, 1H, $J = 8.4$ Hz), 6.90 (d, 1H, $J = 8.4$ Hz), 8.62 (d, 1H, $J = 4.8$ Hz), 8.78 (d, 1H, $J = 4.8$ Hz), 10.86 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.15, 44.29, 44.91, 93.65, 94.89, 98.22, 99.96, 129.73, 131.69, 137.16, 138.35, 138.57, 157.42.

4-Acetamidoquinoline-5,8-dione (10). A solution of 4-acetamido-5,8-dimethoxyquinoline **10c** (5 g, 20.3 mmol) and CAN (22 g, 40.1 mmol) in CH_3CN (50 mL) and H_2O (50 mL) was stirred for 1 h and 45 min at 0 °C. The CH_3CN was evaporated, and a solution of saturated $NaHCO_3$ (500 mL) and H_2O (200 mL) was added and the mixture extracted with $CHCl_3$ (3 × 500 mL). The extract was dried over $MgSO_4$ and concentrated to give the expected product (3.4 g, 77%) as a yellow-brown solid, which was quickly used in the next step. ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 3H), 6.97 (d, 1H, $J = 10.4$ Hz), 7.08 (d, 1H, $J = 10.4$ Hz), 8.83 (d, 1H, $J = 5.6$ Hz), 8.88 (d, 1H, $J = 5.6$ Hz), 11.75 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 26.00, 114.40, 117.75, 138.41, 139.31, 147.34, 148.60, 155.73, 170.53, 182.96, 189.75.

3-Ethylquinolinecarboxylate-5,8-dione (11). 11 was prepared as described for **10** from 3-ethyl-5,8-dimethoxyquinolinecarboxylate[13] (1 g, 3.83 mmol) and CAN (7.4 g, 13.5 mmol) in CH_3CN/H_2O (45 mL/23 mL), and the mixture was stirred for 1 h at room temperature. A saturated solution of $NaHCO_3$ (17 mL) and H_2O (60 mL) was added and the extraction was with CH_2Cl_2 (3 × 100 mL), giving a yellow solid (0.8 g, 91%): mp, 124 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (t, 3H, $J = 7.3$ Hz), 4.49 (q, 2H, $J = 7.3$ Hz), 7.13 (d, 1H, $J = 10.4$ Hz), 7.22 (d, 1H, $J = 10.4$ Hz), 8.99 (d, 1H, $J = 2.2$ Hz), 9.58 (d, 1H, $J = 2.2$ Hz). ^{13}C NMR ($CDCl_3$): δ 14.22, 62.48, 128.62, 129.86, 135.98, 138.29, 139.33, 149.14, 155.17, 163.44, 182.40, 183.63.

6-Hydroxy-4-(2-nitrophenyl)pyrido[3,2-*g*]quinoline-5,-10-dione (12i). A solution of chloro adduct **12a**[15] (1.3 g, 3.56 mmol) and sodium azide (1.3 g, 20.03 mmol) in DMF (11 mL) was heated for 1 h at 90 °C. After the mixture was cooled and concentrated to dryness, a saturated solution of NH_4Cl (20 mL) was added. The mixture was extracted with 5% MeOH/CH_2Cl_2 (5 × 50 mL). The extracts were dried over $MgSO_4$ and concentrated to give **12i** (0.26 g, 21%) as a yellow solid: mp, 180 °C. ^{1}H NMR ($CDCl_3$): δ 7.14 (d, 1H, $J = 5.6$ Hz), 7.26 (dd, 1H, $J = 1.6$ and 8.4 Hz), 7.48 (d, 1H, $J = 4.8$ Hz), 7.70 (ddd, 1H, $J = 1.6$, 8.4 and 7.2 Hz), 7.78 (ddd, 1H, $J = 0.8$, 7.2 and 8.0 Hz), 8.36 (dd, 1H, $J = 1.2$ and 8.0 Hz), 8.81 (d, 1H, $J = 5.6$ Hz), 9.18 (d, 1H, $J = 4.8$ Hz), 11.85 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 115.83, 117.56, 124.99, 126.63, 128.92, 129.71 (2C), 134.17, 134.53, 146.59, 149.24, 149.73, 155.29, 155.38, 156.32, 167.78, 189.23. IR ($CHCl_3$): 3376, 1701, 1649 cm^{-1}. MS (EI mode): *m*/*z* 301 (100), 273 (8). t_R is 2.9 min (98% purity), using system I, and t_R is 1.57 min (97% purity), using system II.

7-Ethyl-4-(2-nitrophenyl)pyrido[3,2-*g*]quinolinecarboxylate-5,10-dione (12j). A solution of 3-ethylquinolinecarboxylate-5,8-dione (0.3 g, 1.29 mmol), 4-(2-nitrophenyl)-1-(dimethylamino)-1-aza-1,3-butadiene (0.33 g, 1.47 mmol), and acetic anhydride (1.4 mL) in CH_3CN (50 mL) was refluxed under nitrogen for 64 h. After the mixture was cooled, the mixture was concentrated to dryness and the residue purified by flash chromatography (CH_2Cl_2/MeOH, 98:2) to give **12j** (53 mg, 10%) as a decomposing brown solid. ^{1}H NMR ($CDCl_3$): δ 1.41 (t, 3H, $J = 7$ Hz), 4.44 (q, 2H, $J = 7$ Hz), 7.28 (dd, 1H, $J = 7.7$ and 1.5 Hz), 7.54 (d, 1H, $J = 4.8$ Hz), 7.72 (dd, 1H, $J = 8.0$ and 1.5 Hz), 7.80 (ddd, 1H, $J = 7.7$, 8.0 and 1.5 Hz), 8.37 (dd, 1H, $J = 8.0$ and 1.5 Hz), 8.97 (d, 1H, $J = 2$ Hz), 9.21 (d, 1H, $J = 4.8$ Hz), 9.65 (d, 1H, $J = 2$ Hz). ^{13}C NMR ($CDCl_3$): δ 14.19, 62.49, 124.91, 127.08, 128.93, 129.74, 129.78, 129.82, 130.23, 134.13, 134.45, 137.05, 146.80, 149.40, 149.85, 149.88, 155.11, 155.99, 163.26, 179.28, 181.96. IR ($CHCl_3$): 1728, 1706, 1680 cm^{-1}. MS (EI mode): *m*/*z* 357 (100), 356 (85), 329 (17), 328 (8). t_R is 6.62 min (98% purity), using system I, and t_R is 6.0 min (96% purity), using system II.

Diels−Alder Reactions of Substituted Quinoline-5,8-diones with 4-(2-Trifluoroacetamidophenyl)-1-dimethyl-1-aza-1,3-butadiene. General Method A. A solution of substituted quinoline-5,8-dione, 4-(2-trifluoroacetamidophenyl)-1-(dimethylamino)-1-aza-1,3-butadiene, and acetic anhydride in CH_3CN was refluxed under nitrogen. After cooling, the mixture was concentrated to dryness and the unreacted starting materials were filtered off over silica gel. Pd/C (10%) and toluene were added to the crude product, and the suspension was refluxed. After cooling, the mixture was concentrated and purified by flash chromatography to give the expected product.

6-Chloro-4-(2-trifluoroacetamidophenyl)pyrido[3,2-*g*]-quinoline-5,10-dione (13a). Method A was used and involved 4-chloroquinoline-5,8-dione **5** (0.7 g, 3.63 mmol), 4-(2-trifluoroacetamidophenyl)-1-(dimethylamino)-1-aza-1,3-butadiene (1.2 g, 4 mmol), acetic anhydride (1 mL), CH_3CN (50 mL), and reflux of 6 h. Filtration was done on silica gel (CH_2Cl_2/MeOH 99.5:0.5). Pd/C (10%, 4 g) and toluene (100 mL) were added, and the solution was refluxed for 2 h. Flash chromatography (CH_2Cl_2/MeOH 98:2) gave **13a** (87 mg, 6%) as a bright-yellow solid: mp, 152 °C. ^{1}H NMR ($CDCl_3$): δ 7.21 (d, 1H, $J = 9$ Hz), 7.46 (dd, 1H, $J = 7.5$ and 7.5 Hz), 7.58 (m, 2H), 7.72 (m, 2H), 7.81 (s, 1H), 8.94 (d, 1H, $J = 5.1$ Hz), 9.13 (d, 1H, $J = 4.7$ Hz). ^{13}C NMR ($CDCl_3$): δ 115.49 (q, $J = 287.2$ Hz), 125.55, 128.11, 128.27, 129.20, 129.35, 130.16, 131.02, 131.32, 131.52, 133.58, 145.13, 147.57, 148.24, 149.85, 154.35, 154.86, 155.34 (q, $J = 37$ Hz), 179.28, 182.40. IR ($CHCl_3$): 3410, 1737, 1707, 1648, 1602 cm^{-1}. MS (EI mode): *m*/*z* 357 (100), 356 (85), 329 (17), 328 (8). t_R is 4.99 min (97% purity), using system I, and t_R is 3.34 min (95% purity), using system II.

6-Methoxy-4-(2-trifluoroacetamidophenyl)pyrido[3,2-*g*]quinoline-5,10-dione (13b). Method A was used and involved 4-methoxyquinoline-5,8-dione **6** (3.5 g, 16 mmol), 4-(2-trifluoroacetamidophenyl)-1-(dimethylamino)-1-aza-1,3-butadiene (7 g, 24 mmol), acetic anhydride (15 mL), CH_3CN (200 mL), and reflux of 18 h. Filtration was done on silica gel (CH_2Cl_2/MeOH 99.5:0.5). Pd/C (10%, 6 g) and toluene (150 mL) were added, and the solution was refluxed for 2 h. Flash chromatography (CH_2Cl_2/MeOH 95:5) gave **13b** (0.6 g, 9%) as a pale-green solid: mp, 158 °C. ^{1}H NMR ($CDCl_3$): δ 4.04 (s, 3H), 7.22 (d, 1H, J = 6 Hz), 7.24 (dd, 1H, J = 1.6 and 7.6 Hz), 7.46 (dd, 1H, J = 7.6 and 7.2 Hz), 7.56 (d, 1H, J = 4.8 Hz), 7.60 (dd, 1H, J = 7.6 and 7.2 Hz), 7.79 (d, 1H, J = 8.4 Hz), 7.97 (s, 1H), 8.95 (d, 1H, J = 6 Hz), 9.13 (d, 1H, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$): δ 65.84, 111.53, 115.34 (q, J = 305 Hz), 120.60, 125.44, 127.82, 129.09, 129.46, 129.80, 131.28, 131.70, 133.83, 147.15, 148.22, 150.08, 154.29, 155.09 (q, 42 Hz), 156.07, 165.95, 180.26, 183.00. IR ($CHCl_3$): 3399, 1734, 1706, 1675, 1584 cm^{-1}. MS (EI mode): m/z 427 (100), 358 (36), 315 (71). t_R is 3.87 min (98% purity), using system I, and t_R is 2.21 min (90% purity), using system II.

6-Bromo-4-(2-trifluoroacetamidophenyl)pyrido[3,2-*g*]-quinoline-5,10-dione (13d). Method A was used and involved 4-bromoquinoline-5,8-dione **8** (2 g, 8.4 mmol), 4-(2-trifluoroacetamidophenyl)-1-(dimethylamino)-1-aza-1,3-butadiene (3.6 g, 12.6 mmol), silica gel (5 g) instead of acetic anhydride, CH_3CN (220 mL), and reflux of 10 h. Filtration was done on silica gel (CH_2Cl_2/MeOH 99.5:0.5). Pd/C (10%, 2.1 g) and toluene (21 mL) were added, and the solution was refluxed for 4 h. Flash chromatography (CH_2Cl_2/MeOH 98:2) gave **13d** (0.19 g, 5%) as a beige solid: mp, 145 °C. ^{1}H NMR ($CDCl_3$): δ 7.21 (dd, 1H, J = 7.2 and 1.2 Hz), 7.44 (ddd, 1H, J = 7.2, 7.2, and 1.2 Hz), 7.55 (ddd, 1H, J = 7.2, 7.2, and 1.2 Hz), 7.57 (d, 1H, J = 4.8 Hz), 7.69 (dd, 1H, J = 7.2 and 1.2 Hz), 7.91 (d, 1H, J = 4.8 Hz), 8.20 (br s, 1H), 8.73 (d, 1H, J = 4.8 Hz), 9.05 (d, 1H, J = 4.8 Hz). ^{13}C NMR ($CDCl_3$): δ 115.12 (q, J = 287.1 Hz), 125.41, 127.71, 128.94, 129.30, 129.30, 129,72, 130.91, 131.10, 132.89, 133.31, 134.26, 147.35, 147.79, 149.24, 153.44, 154.30, 154.78 (q, J = 36.8 Hz), 178.86, 182.05. IR ($CHCl_3$): 3401, 1735, 1706, 1684, 1603 cm^{-1}. MS (EI mode): m/z 477 (34), 474 (35), 408 (21), 406 (25), 365 (76), 362 (100). t_R is 5.21 min (92% purity), using system I, and t_R is 3.66 min (95% purity), using system II.

4-(2-Trifluoroacetamidophenyl)pyrido[3,2-*g*]quinoline-5,10-dione (13e). Method A was used and involved quinoline-5,8-dione **4** (1 g, 6.3 mmol), 4-(2-trifluoroacetamidophenyl)-1-(dimethylamino)-1-aza-1,3-butadiene (3.59 g, 12.6 mmol), acetic anhydride (7.5 mL), CH_3CN (175 mL), and reflux of 24 h. Filtration on silica gel (CH_2Cl_2/MeOH 95:5). Pd/C (10%, 6.2 g) and toluene (150 mL) were added, and the solution was refluxed for 12 h. Flash chromatography (CH_2Cl_2/MeOH 95:5) gave **13e** (125 mg, 5%) as a yellow solid: mp, 205 °C. ^{1}H NMR ($CDCl_3$): δ 7.20 (dd, 1H, J = 8 and 1.2 Hz), 7.46 (ddd, 1H, J = 8.0, 8.0, and 1.2 Hz), 7.58 (d, 1H, J = 4.4 Hz), 7.59 (ddd, 1H, J = 8.0, 8.0, and 1.2 Hz), 7.74 (m, 3H), 8.42 (dd, 1H, J = 8.0 and 1.6 Hz), 9.14 (dd, 1H, J = 4.4 and 1.6 Hz), 9.16 (d, 1H, J = 4.4 Hz). ^{13}C NMR ($CDCl_3$): δ 115.46 (q, J = 291 Hz), 125.26, 127.45, 127.94, 128.54, 129.01, 129.99, 130.49, 131.15, 131.50, 133.90, 135.57, 147.84, 147.99, 149.68, 154.93, 155.39 (q, J = 40 Hz), 155.86, 179.66, 183.19. IR ($CHCl_3$): 3420, 1740, 1706, 1679, 1587 cm^{-1}. MS (EI mode): m/z 397 (97), 382 (27), 328 (48), 285 (100). t_R is 4.20 min (92% purity), using system I, and t_R is 2.35 min (99% purity), using system II.

6-Nitro-4-(2-trifluoroacetamidophenyl)pyrido[3,2-*g*]-quinoline-5,10-dione (13f). Method A was used and involved 4-nitroquinoline-5,8-dione **9** (1.5 g, 7.35 mmol), 4-(2-trifluoroacetamidophenyl)-1-(dimethylamino)-1-aza-1,3-butadiene (4.2 g, 14.7 mmol), acetic anhydride (7.5 mL), CH_3CN (100 mL), and reflux of 18 h. Filtration was done on silica gel (CH_2Cl_2/MeOH 95:5). Pd/C (10%, 2.5 g) and toluene (30 mL) were added, and the solution was refluxed for 5 h. Flash chromatography (CH_2Cl_2/MeOH 95:5) gave **13f** (0.4 g, 13%) as a beige solid: mp, 158 °C. ^{1}H NMR ($CDCl_3$): δ 7.34 (d, 1H, J = 7.2 Hz), 7.45 (d, 1H, J = 7.2 Hz), 7.54 (m, 2H), 7.69 (d, 1H, J = 4.4 Hz), 7.77 (d, 1H, J = 4.4 Hz), 8.04 (s, 1H), 9.18 (d, 1H, J = 4.4 Hz), 9.32 (d, 1H, J = 4.4 Hz). ^{13}C NMR ($CDCl_3$): δ 115.41 (q, J = 287 Hz), 120.85, 122.58, 126.51, 128.16, 129.25, 129.33, 130.39, 130.64, 131.42, 134.24, 148.11, 148.18, 149.11, 154.51, 155.21 (q, 37 Hz), 155.77, 157.13, 177.79, 180.54. IR ($CHCl_3$): 3400, 1740, 1707, 1648, 1601 cm^{-1}. MS (EI mode): m/z 442 (7), 330 (12), 284 (40). t_R is 5.46 min (99% purity), using system I, and t_R is 4.31 min (96% purity), using system II.

6-(Acetamido)-4-(2-trifluoroacetamidophenyl)pyrido-[3,2-*g*]quinoline-5,10-dione (13h). Method A was used and involved 4-acetamidoquinoline-5,8-dione **10** (3.4 g, 15.7 mmol), 4-(2-trifluoroacetamidophenyl)-1-(dimethylamino)-1-aza-1,3-butadiene (5 g, 17.5 mmol), acetic anhydride (6 mL), CH_3CN (270 mL), 10% Pd/C (4.4 g), and reflux of 15 h. Flash chromatography ($CHCl_3$/MeOH 98:2) gave **13h** (80 mg, 1%) as a brown solid: mp, >260 °C. ^{1}H NMR ($CDCl_3$): δ 2.23 (s, 3H), 7.25 (dd, 1H, J = 7.7 and 1.5 Hz), 7.48 (ddd, 1H, J = 7.3, 7.7, and 1.1 Hz), 7.53 (dd, 1H, J = 4.8 Hz), 7.60 (ddd, 1H, J = 7.7, 7.3, and 1.5 Hz), 7.78 (d, 1H, J = 7.3 Hz), 7.93 (br s, 1H), 8.86 (d, 1H, J = 5.7 Hz), 8.93 (d, 1H, J = 5.7 Hz), 9.08 (d, 1H, J = 4.8 Hz). ^{13}C NMR ($CDCl_3$): δ 25.20, 115.63 (q, J = 288 Hz), 115.93, 116.66, 126.61, 127.86, 128.75, 129.11, 129.28, 131.31, 131.62, 134.87, 146.17, 146.82, 148.33, 149.60, 154.30, 154.82, 154.94 (q, J = 36 Hz), 170.13, 179.34, 186.80. IR ($CHCl_3$): 3410, 3277, 1719, 1707, 1702 cm^{-1}. MS (EI mode): m/z 454 (9), 412 (7), 343 (100), 300 (17). t_R is 3.83 min (96% purity), using system I, and t_R is 2.56 min (93% purity), using system II.

7-Ethyl-4-(2-trifluoroacetamidophenyl)pyrido[3,2-*g*]-quinolinecarboxylate-5,10-dione (13j). Method A was used and involved 3-ethylquinolinecarboxylate-5,8-dione **11** (0.45 g, 1.94 mmol), 4-(2-trifluoroacetamidophenyl)-1-(dimethylamino)-1-aza-1,3-butadiene (0.61 g, 2.14 mmol), acetic anhydride (1.4 mL), CH_3CN (50 mL), and reflux of 24 h. Filtration was done on silica gel (CH_2Cl_2/ MeOH 98:2), MnO_2 (0.63 g), $CHCl_3$ (20 mL), room temperature, 3 h. Flash chromatography (CH_2Cl_2) gave **13j** (27 mg, 3%) as a brown solid: mp, 124 °C. ^{1}H NMR ($CDCl_3$): δ 1.42 (t, 3H, J = 7 Hz), 4.45 (q, 2H, J = 7 Hz), 7.22 (dd, 1H, J = 7.7 and 1.5 Hz), 7.47 (ddd, 1H, J = 7.7, 7.7, and 0.8 Hz), 7.61 (ddd, 1H, J = 7.7, 7.7, and 1.5 Hz), 7.63 (d, 1H, J = 4.8 Hz), 7.68 (br s, 1H), 7.74 (d, 1H, J = 8.1 Hz), 9.00 (d, 1H, J = 1.9 Hz), 9.20 (d, 1H, J = 4.8 Hz), 9.65 (d, 1H, J = 1.9 Hz). ^{13}C NMR ($CDCl_3$): δ 14.11, 62.56, 115.46 (q, J = 287 Hz), 125.73, 127.50, 127.99, 129.01, 129.97 (2), 130.23, 131.27, 131.43, 134.16, 136.91 (2), 148.32, 149.53, 154.88, 155.57 (q, J = 38 Hz), 155.76, 163.11, 179.05, 182.10. IR ($CHCl_3$): 3401, 1730, 1709, 1681 cm^{-1}. MS (EI mode): m/z 469 (17), 468 (6), 357 (100), 356 (81), 329 (17), 328 (6). t_R is 6.62 min (98% purity), using system I, and t_R is 6.00 min (96% purity), using system II.

8-Oxo-4-(2-trifluoroacetamidophenyl)pyrido[3,2-*g*]quinoline-5,10-dione (13k). Method A was used and involved 5,8-dioxocarbostyril **7** (1.04 g, 5.9 mmol), 4-(2-trifluoroacetamidophenyl)-1-(dimethylamino)-1-aza-1,3-butadiene (1.9 g, 6.5 mmol), acetic anhydride (2.2 mL), 10% Pd/C (1.6 g), CH_3CN (500 mL), and reflux of 15 h. Flash chromatography (CH_2Cl_2/MeOH 95:5) gave **13k** (0.4 g, 16%) as a yellow solid: mp, >260 °C. ^{1}H NMR ($CDCl_3$): δ 6.90 (dd, 1H, J = 9.9 Hz), 7.19 (dd, 1H, J = 7.8 and 1.5 Hz), 7.47 (ddd, 1H, J = 7.8, 7.8, and 1.1 Hz), 7.60 (m, 2H), 7.68 (m, 2H), 7.93 (d, 1H, J = 9.9 Hz), 9.09 (d, 1H, J = 4.8 Hz). ^{13}C NMR ($CDCl_3$): δ 115.5 (q, J = 288 Hz), 126.34, 126.90, 127.08, 128.69, 129.12, 130.89, 134.61, 135.63, 146.85, 148.02, 150.56, 151.70, 152.83, 154.92, 155.57 (q, J = 38 Hz), 161.92, 169.63, 179.50, 181.13. IR ($CHCl_3$): 3401, 3334, 1735, 1685, 1664 cm^{-1}. MS (EI mode): m/z 413 (33), 344 (17), 301 (100), 177 (44). t_R is 3.56 min (92% purity), using system I, and t_R is 2.02 min (97% purity), using system II.

6-Amino-4-(2-trifluoroacetamidophenyl)pyrido[3,2-*g*]-quinoline-5,10-dione (13c). A solution of chloro adduct **13a** (0.64 g, 1.48 mmol) and sodium azide (0.27 g, 8.4 mmol) in DMF (12 mL) was heated for 4 h at 90 °C. After the mixture was cooled and concentrated to dryness, $CHCl_3$ (1 L) was added

and the mixture was stirred for 12 h. The mixture was concentrated and the residue purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to give **13c** (37 mg, 6%) as a yellow solid: mp, >260 °C. ^{1}H NMR (DMSO-d_6): δ 5.75 (s, 2H), 7.24 (d, 1H, J = 5.8 Hz), 7.72−7.82 (m, 4H), 7.82 (d, 1H, J = 5.0 Hz), 8.61 (d, 1H, J = 5.8 Hz), 9.31 (d, 1H, J = 5.0 Hz), 10.78 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 112.65, 114.61, 115.86 (q, J = 286 Hz), 118.30, 126.37, 127.37, 128.60, 129.20, 131.18, 131.51, 136.10, 146.89, 148.50, 150.09, 151.48, 153.18, 154.93 (q, J = 42.8 Hz), 155.25, 180.39, 184.89. IR ($CHCl_3$): 3410, 3351, 1711, 1652, 1606 cm^{-1}. MS (EI mode): m/z 412 (10), 343 (66), 300 (100). t_R is 3.78 min (95% purity), using system I, and t_R is 2.05 min (95% purity), using system II.

6-(Dimethylamino)-4-(2-trifluoroacetamidophenyl)-pyrido[3,2-*g*]quinoline-5,10-dione (13g) and 12-(Dimethylamino)benzo[*b*]pyrido[4,3,2-*de*][1,7]phenanthroline-8-one (14g). Dimethylamine hydrochloride (0.37 g, 4.6 mmol) and NaOH (0.18 g, 4.6 mmol) were successively added to a solution of chloro adduct **13a** (0.5 g, 1.15 mmol) in a mixture H_2O/THF (12 mL/25 mL). The mixture was heated at 60 °C for 3 h and concentrated to dryness. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 98:2) to give successively **13g** and **14g**.

13g: yield of 70 mg, 14%, orange solid, mp 150 °C. ^{1}H NMR ($CDCl_3$): δ 2.75 (s, 6H), 6.94 (d, 1H, J = 6 Hz), 7.07 (dd, 1H, J = 7.7 and 1.5 Hz), 7.32 (ddd, 1H, J = 7.7, 7.7, and 0.7 Hz), 7.49 (d, 1H, J = 5 Hz), 7.55 (ddd, 1H, J = 7.7, 7.7, and 1.7 Hz), 7.85 (d, 1H, J = 7.7 Hz), 8.29 (br s, 1H), 8.55 (d, 1H, J = 6 Hz), 9.05 (d, 1H, J = 5 Hz). ^{13}C NMR ($CDCl_3$): δ 43.21, 112.58, 115.58 (q, J = 287 Hz), 119.13, 125.29, 126.72, 129.03, 129.60, 130.30, 131.13, 132.77, 133.28, 146.18, 147.82, 149.71, 151.05, 153.28, 154.76, 155.76 (q, J = 37 Hz), 180.48, 183.96. IR (KBr): 3175, 1724, 1701, 1654 cm^{-1}. MS (EI mode): m/z 440 (100), 353 (15), 69 (84). t_R is 4.85 min (98% purity), using system I, and t_R is 2.78 min (95% purity), using system II.

14 g: yield of 40 mg, 11%, red solid, mp 238 °C. ^{1}H NMR ($CDCl_3$): δ 3.10 (s, 6H), 7.10 (d, 1H, J = 5.5 Hz), 7.73 (dd, 1H, J = 6.8 and 6.8 Hz), 7.87 (dd, 1H, J = 6.8 and 6.8 Hz), 8.22 (d, 1H, J = 6.8 Hz), 8.54 (m, 2H), 8.59 (d, 1H, J = 5.5 Hz), 9.26 (d, 1H, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$): δ 44.07, 113.72, 117.93, 119.68, 120.41, 122.89, 128.44, 130.47, 131.74, 137.82, 145.29, 146.53, 149.38, 150.06, 150.32, 151.18, 156.85, 181.72. IR (KBr): 1690 cm^{-1}. MS (EI mode): m/z 326 (44), 311 (100), 254 (14). t_R is 6.45 min (91% purity), using system I, and t_R is 2.91 min (92% purity), using system II.

Formation of the Pentacyclic Compounds. General Method B. Compound **13** was dissolved in a mixture of CH_2Cl_2−CF_3COOH, and the mixture was refluxed for different periods. After the mixture was cooled and concentrated to dryness, 1 N NaOH and $CHCl_3$ were added and the mixture was stirred overnight. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue on a flash chromatography column gave the expected product.

General Method C. Compound **13** was dissolved in a mixture of $CHCl_3$ and 1 N NaOH, and the mixture was stirred at room temperature for 1 h. The organic layer was separated and the aqueous layer extracted with $CHCl_3$. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue on a flash chromatography column gave the expected product.

12-Chlorobenzo[*b*]pyrido[4,3,2-*de*][1,7]phenanthrolin-8-one (14a). Method B was used and involved **13a** (100 mg, 0.232 mmol), TFA (1 mL) in CH_2Cl_2 (10 mL), reflux of 30 min, and 1 N NaOH (6 mL) in $CHCl_3$ (6 mL). Flash chromatography (CH_2Cl_2/MeOH 97:3) gave **14a** (64 mg, 87%) as a yellow solid: mp >260 °C. ^{1}H NMR ($CDCl_3$): δ 7.77 (d, 1H, J = 4.8 Hz), 7.80 (dd, 1H, J = 8 and 8 Hz), 7.89 (dd, 1H, J = 8 and 8 Hz), 8.25 (d, 1H, J = 8 Hz), 8.51 (d, 1H, J = 8 Hz), 8.61 (d, 1H, J = 5.6 Hz), 8.84 (d, 1H, J = 4.8 Hz), 9.28 (d, 1H, J = 5.6 Hz). ^{13}C NMR ($CDCl_3$): δ 118.06, 120.19, 120.79, 122.65, 129.42, 129.95, 131.66, 131.77, 132.01, 137.88, 144.71, 144.73, 145.78, 147.01, 149.77, 150.27, 151.22, 179.88. IR ($CHCl_3$): 1694, 1601 cm^{-1}. MS (EI mode): m/z 319 (100), 317(28), 291 (100), 289 (32), 254 (26). t_R is 6.8 min (98% purity), using system I, and t_R is 5.54 min (98% purity), using system II.

12-Bromobenzo[*b*]pyrido[4,3,2-*de*][1,7]phenanthrolin-8-one (14d). Method C was used and involved **13d** (20 mg, 0.045 mmol) and 1 N NaOH (0.6 mL) in $CHCl_3$ (13 mL). Flash chromatography on silica gel (CH_2Cl_2/MeOH 98:2) gave **14d** (8.1 mg, 55%) as a yellow solid: mp >260 °C. ^{1}H NMR ($CDCl_3$): δ 7.26 (d, 1H, J = 5.6 Hz), 7.87 (ddd, 1H, J = 8.4, 8.4, and 1.2 Hz), 7.97 (ddd, 1H, J = 8.4, 8.4, and 1.2 Hz), 8.23 (dd, 1H, J = 8.4 and 1.2 Hz), 8.64 (dd, 1H, J = 8.4 and 1.2 Hz), 8.67 (d, 1H, J = 5.6 Hz), 8.79 (d, 1H, J = 5.6 Hz), 9.39 (d, 1H, J = 5.6 Hz). ^{13}C NMR ($CDCl_3$): δ 118.09, 120.19, 121.01, 122.77, 129.93, 130.48, 131.50, 131.87, 132.44, 135.62, 138.01, 144.62, 146.04, 146.70, 149.72, 150.37, 151.05, 180.06. IR ($CHCl_3$): 1686, 1601 cm^{-1}. MS (EI mode): m/z 328 (4), 327 (14), 312 (82), 283 (28), 255 (100). t_R is 7.37 min (98% purity), using system I, and t_R is 6.41 min (95% purity), using system II.

Benzo[*b*]pyrido[4,3,2-*de*][1,7]phenanthrolin-8-one (14e). Method B was used and involved **13e** (30 mg, 0.076 mmol), TFA (1 mL) in CH_2Cl_2 (2 mL), reflux of 3 h, and 1 N NaOH (2 mL) in $CHCl_3$ (2 mL). **14e** was obtained (17 mg, 80%) as a yellow solid: mp >260 °C. ^{1}H NMR ($CDCl_3$): δ 7.94 (dd, 1H, J = 4.4 and 8 Hz), 7.97 (dd, 1H, J = 8.2 and 8.2 Hz), 8.10 (dd, 1H, J = 8.2 and 8.2 Hz), 8.48 (d, 1H, J = 8.2 Hz), 8.76 (d, 1H, J = 8.2 Hz), 8.80 (d, 1H, J = 5.2 Hz), 9.23 (dd, 1H, J = 1.6 and 4.4 Hz), 9.49 (d, 1H, J = 5.2 Hz), 9.50 (dd, 1H, J = 1.6 and 8 Hz). ^{13}C NMR ($CDCl_3$): δ 118.06, 119.82, 121.88, 123.02, 128.07, 129.33, 131.44, 131.98, 132.98, 134.17, 138.08, 145.62, 147.27, 147.50, 147.76, 150.46, 153.18, 180.94. IR ($CHCl_3$): 1690, 1604 cm^{-1}. MS (EI mode): m/z 283 (100), 255 (51). t_R is 5.75 min (98% purity), using system I, and t_R is 2.92 min (98% purity), using system II.

12-Nitrobenzo[*b*]pyrido[4,3,2-*de*][1,7]phenanthrolin-8-one (14f). Method C was used and involved **13f** (20 mg, 0.045 mmol) and 1 N NaOH (0.6 mL) in $CHCl_3$ (13 mL). Flash chromatography on silica gel (CH_2Cl_2/MeOH 98:2) gave **14f** (8.1 mg, 55%) as a yellow solid: mp >260 °C. ^{1}H NMR (DMSO-d_6): δ 7.56 (d, 1H, J = 6.0 Hz), 7.89 (dd, 1H, J = 8.1 and 6.0 Hz), 8.02 (dd, 1H, J = 8.1 and 6.4 Hz), 8.20 (d, 1H, J = 8.0 Hz), 8.79 (d, 1H, J = 6.4 Hz), 8.94 (d, 1H, J = 8.0 Hz), 9.10 (d, 1H, J = 5.1 Hz), 9.28 (d, 1H, J = 5.1 Hz). ^{13}C NMR (DMSO-d_6): δ 120.45, 120.74, 123.65, 123.78, 125.42, 129.14, 130.79, 131.82, 137.04, 144.88, 149.62, 151.83, 153.57, 153.68, 156.85, 162.28, 164.75, 178.09. IR ($CHCl_3$): 1688, 1601 cm^{-1}. t_R is 5.33 min (97% purity), using system I, and t_R is 3.54 min (92% purity), using system II.

11-Ethylbenzo[*b*]pyrido[4,3,2-*de*][1,7]phenanthroline-carboxylate-8-one (14j). A suspension of nitro derivative **12j** (60 mg, 0.15 mmol) and 10% Pd/C (48 mg) in MeOH (10 mL) was fitted into a hydrogenation apparatus (1 atm) and stirred for 2 h under a hydrogen atmosphere. The mixture was concentrated and the residue purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to give **14j** (33 mg, 63%) as a yellow solid: mp >260 °C. ^{1}H NMR ($CDCl_3$): δ 1.53 (t, 3H, J = 7.3 Hz), 4.57 (q, 2H, J = 7.3 Hz), 7.86 (ddd, 1H, J = 8.0, 8.0, and 1.1 Hz), 7.99 (ddd, 1H, J = 8.0, 8.4, and 1.1 Hz), 8.42 (d, 1H, J = 8.4 Hz), 8.64 (d, 1H, J = 8.0 Hz), 8.70 (d, 1H, J = 5.5 Hz), 9.37 (d, 1H, J = 5.5 Hz), 9.59 (d, 1H, J = 2.2 Hz), 9.90 (d, 1H, 2.2 Hz). ^{13}C NMR ($CDCl_3$): δ 13.01, 60.97, 116.85, 118.69, 120.56, 121.68, 128.30, 128.49, 130.31, 130.79, 131.29, 134.52, 136.82, 144.27, 145.31, 145.83, 148.13, 149.29, 151.91, 162.90, 178.99. IR ($CHCl_3$): 1726, 1693 cm^{-1}. MS (EI mode): m/z 355 (100). t_R is 8.19 min (94% purity), using system I, and t_R is 8.94 min (98% purity), using system II.

10-Hydroxybenzo[*b*]pyrido[4,3,2-*de*][1,7]phenanthroline-8-one (14k). Method C was used and involved a solution of **13k** (180 mg, 0.44 mmol) and 1 N NaOH (22 mL) in $CHCl_3$ (70 mL) that was stirred overnight. CH_3COOH was added until neutralization, and the organic layer was separated and extracted with $CHCl_3$/MeOH 95:5 (3 × 50 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated to give **14k** (110 mg, 85%) as a purple solid: mp >260 °C. ^{1}H

NMR (DMSO-d_6): δ 6.91 (d, 1H, J = 9.2 Hz), 7.84 (d, 1H, J = 7.8 and 8.8 Hz), 7.97 (dd, 1H, J = 7.8 and 7.8 Hz), 8.21 (d, 1H, J = 7.8 Hz), 8.71 (d, 1H, J = 9.2 Hz), 8.87 (d, 1H, J = 8.8 Hz), 9.04 (d, 1H, J = 5.5 Hz), 9.27 (d, 1H, J = 5.5 Hz). IR (KBr): 1664, 1608 cm^{-1}. MS (EI mode): *m/z* 299 (100), 298 (66), 271 (17), 270 (13), 243 (39), 242 (29). t_R is 4.16 min (96% purity), using system I, and t_R is 2.66 min (93% purity), using system II.

2. Pharmacology. 2.1. 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 were 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 40 000 cells/mL of 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.[19,20] This assessment of cell population growth is based on the capability of living cells to reduce the yellow reagent 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 wavelength of 570 nm (with a reference at 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^{-10} M were assayed for each of the 25 drugs under study (see Table 2).

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

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