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Bioorganic & Medicinal Chemistry 12 (2004) 6505–6515
Bioorganic & Medicinal Chemistry

Synthesis and biological evaluation of new 1,5-diazaanthraquinones with cytotoxic activity

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Received 3 August 2004; revised 10 September 2004; accepted 14 September 2004
Available online 6 October 2004

Abstract—A series of 1,5-diazaanthraquinone derivatives was synthesized and their in vitro cytotoxic activities were evaluated against several human cancer cell lines. The 1,5-diazaanthraquinone chromophore has been synthesized either on the basis of hetero Diels–Alder reactions involving different quinoline-5,8-diones and α,β-unsaturated aldehyde *N*,*N*-dimethylhydrazones or by thermolysis of different arylaminomethylene Meldrum's acid derivatives. Some of these compounds showed cytotoxic activity comparable to that of mitoxantrone against most of the cell lines tested. Compounds **20**, **30**, **31** and **37** were 4–54 times more potent that mitoxantrone against A549, H116, PSN1 and T98G cancer cell lines but, interestingly, they were 3–16 times less potent against the human breast carcinoma SKBR3. Some structure–activity relationships are described, the most significant one being the increase in cytotoxicity resulting from the introduction of a halogen atom at the C-4 position.

1. Introduction

Products containing an anthracene-9,10-dione substructure are an important class of antitumour compounds. They include the anthracyclines,[1] as mitoxantrone (Fig. 1), the pluramycins[2] and some of the enediyne antibiotics.[3] At least in the case of the anthracyclines, the antitumour activity is mainly attributed to two mechanisms of action. Because of their planar chromophore, they are able to insert between two base pairs in the DNA helix[4] causing a local untwisting of the helix that results in topoisomerase II inhibition,[5] miscoding and possible cell death.[6] Additionally, their antitumour activity may be associated to the formation of DNA damaging anion-radical intermediates by reduction of the quinone unit.[7] The generation of reactive oxygen species also has a role in modulation of angiogenesis by the anthracyclines.[8]

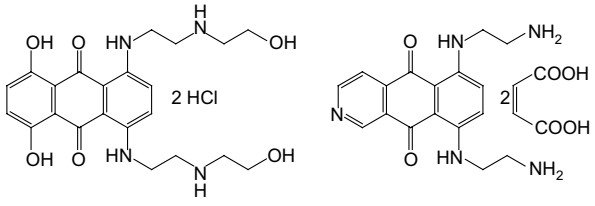

Figure 1.

Isosteric substitution of one or more carbons of the benzene rings by nitrogen atoms should afford compounds with geometries similar to those of the parent structures, but with increased affinity for DNA due to the presence of sites suitable for hydrogen bonding or ionic interactions. Also, the electron-withdrawing properties of the heterocyclic rings would facilitate the formation of anion radicals. Based on this idea, some aza bioisosteres related to the anthracene-9,10-diones have been synthesized and screened in vitro and in vivo against a wide spectrum of tumour cell lines.[9] Among them, pixantrone (Fig. 1) shows promise for development as anticancer agent, currently being in phase III trials for the

Keywords: Antitumour compounds; Cytotoxicity; 1,5-Diazaanthraquinones; Heterocyclic quinones.
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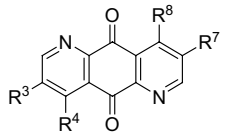
doi:10.1016/j.bmc.2004.09.021



1

Figure 2.

treatment of non-Hodgking's lymphoma.[10] Interestingly, this compound showed an antitumour activity comparable to that of mitoxantrone and doxorubicin but it showed no measurable cardiotoxicity compared to them at equi-effective doses in animal models.[11]

Although the considerations outlined above regarding bioisosteric replacement of carbon by nitrogen would apply particularly well to diaza derivatives, these compounds have received little attention and most of the published studies are focused on 1,8-diazaanthraquinones.[12] 1,5-Diazaanthraquinones have been mainly used as intermediates in the synthesis of pyridoacridines[13] but their potential as antitumour agents is almost unexplored.[12a,14] Recently we described in a preliminary communication[15] the synthesis and the activity of a small series of 1,5-diazaanthraquinones. Herein, we report in full the synthesis and biological evaluation of 1,5-diazaanthraquinone derivatives, with general structure **1** (Fig. 2), bearing different substituents to explore their influence on the cytotoxic activity of this type of diazaanthraquinone chromophore.

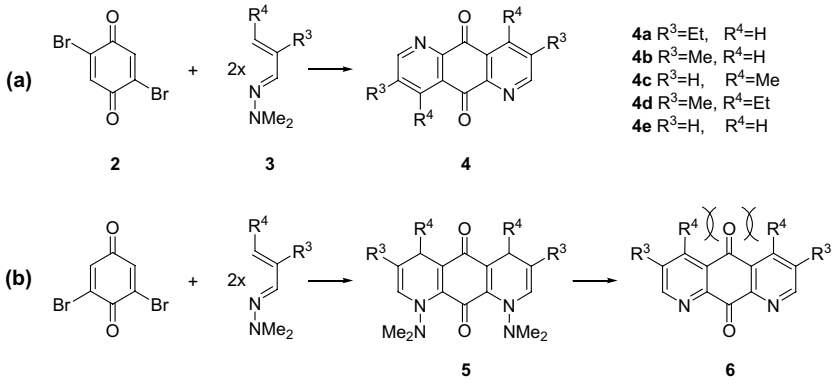
2. Results and discussion

2.1. Chemistry

Symmetrically substituted derivatives of the 1,5-diazaanthraquinone system (Scheme 1a) were prepared in a single step using a strategy based on the double regioselective hetero Diels–Alder reaction.[16] Thus, 2,5-dibromobenzoquinone **2** was treated with the corresponding 1-dimethylamino-1-azadienes **3** to give compounds **4**, in 68–89% yields. It is worthy of note that the behaviour of quinone **2** towards 4-substituted 1-dimethylamino-1-azadienes was different to the one observed for 2,6-dibromobenzoquinone, where the Diels–Alder products were compounds **5** and their aromatization to **6** had to be forced by pyrolysis (Scheme 1b).[16a,b] This difference could be attributed to steric compression of compounds **6**, which does not exist in their regioisomers **4**.

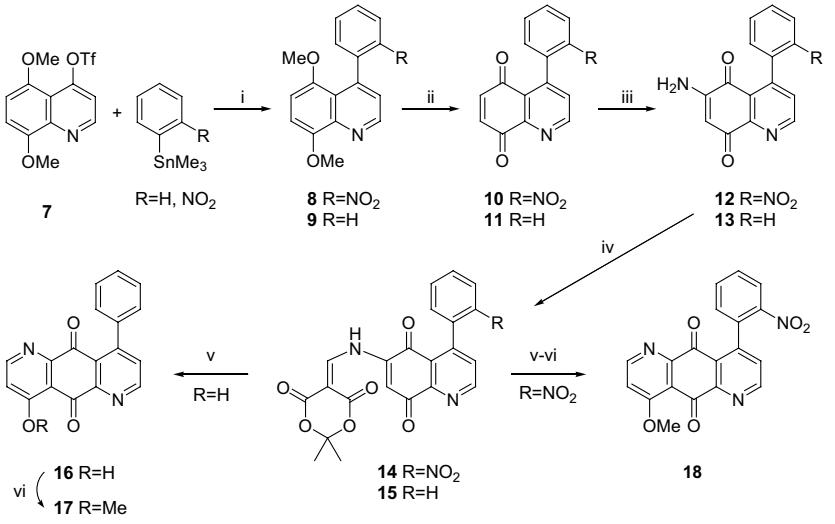
A Stille coupling was used to obtain some 4-aryl substituted derivatives (compounds **16**–**18**, Scheme 2) by a route similar to that used in the synthesis of a regioisomer of the marine alkaloid meridine.[14] Thus, Stille coupling of the previously reported triflate **7**[17] with the known 2-nitrophenyltrimethylstannane[18] catalyzed by $Pd(PPh_3)_4$ afforded compound **8** in 84% yield. Oxidative demethylation of **8** with cerium(IV) ammonium nitrate in acetonitrile–sulfuric acid afforded the quinoline-5,8-dione **10** in 52% yield. The known quinoline **9** and quinoline-5,8-dione **11**[19] were obtained by the same strategy. Aminoquinoline-5,8-diones **12** and **13** were obtained by conjugate addition of hydrazoic acid to **10** and **11**, respectively, in the conditions reported by Couladouros et al.[20] The addition was regiospecific, as expected, because it is controlled by the position of the nitrogen atom of the quinoline-5,8-dione.[21] Amines **12** and **13** were transformed into compounds **14** and **15** by treatment with Meldrum's acid and triethyl orthoformate. The thermolysis of **15** gave quantitatively **16**, which by treatment with diazomethane gave **17** in 19% yield. The known **18**[13b] was obtained in 17% overall yield by the same two-step procedure.

Treatment of quinoline-5,8-dione derivative **19**[13c] with 3-methyl-1-dimethylamino-1-azadiene at room temperature afforded the fully aromatic derivative **20** in 80% yield (Scheme 3), while a similar reaction of **19** with 4-(2-nitrophenyl)-1-dimethylamino-1-azadiene afforded the known aromatic derivative **21**[13b,14] only when the mixture was sonicated at 50 °C for 125 h. On the other hand, use of 4-methyl-1-dimethylamino-1-azadiene led to compound **22**, arising from elimination of hydrobromic acid from the primary Diels–Alder adduct, which was aromatized by elimination of dimethylamine under thermal conditions to give compound **23**. Treatment of

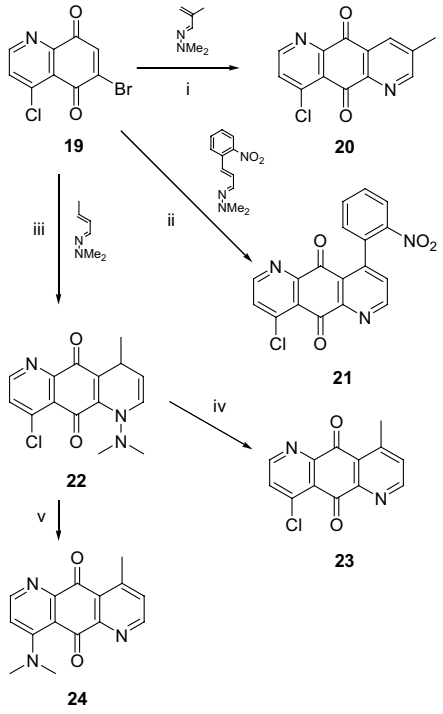


2 3 4 5 6

Scheme 1.



Scheme 2. Reagents and conditions: (i) $Pd(PPh_3)_4$, CuBr, 1,4-dioxane, 90 °C, 1 h 30 min; (ii) $(NH_4)_2Ce(NO_3)_6$, CH_3CN/H_2SO_4 (2 M), rt, 3 h; (iii) NaN_3, MeOH/HCl, rt, 3 h 30 min; (iv) Meldrum's acid, $HC(OEt)_3$, reflux, 6 h; (v) Ph_2O, 200–220 °C, 15 min; (vi) diazomethane, CH_2Cl_2/MeOH, rt, 10 min.



Scheme 3. Reagents and conditions: (i) Et_2O/CH_3CN, rt, 16 h; (ii) $CHCl_3$, ultrasound, 50 °C, 125 h; (iii) Et_2O/CH_3CN, rt, 22 h; (iv) 110 °C, 0.1 Torr, 2 h; (v) THF/HCl (6 N), 80 °C, 1 h.

compound **22** with dilute HCl led to aromatization with concomitant reaction of the liberated dimethylamine at the C-8 position, affording compound **24**.

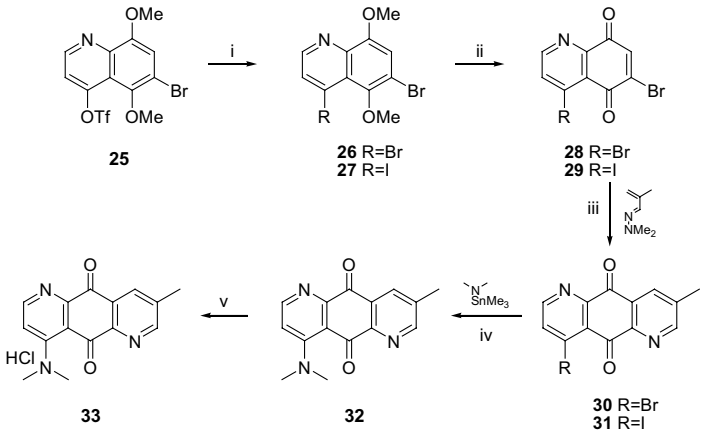
The nucleophilic substitution of triflate **25**, which was obtained as previously described,[22] with excess of LiBr or LiI in dioxane under reflux gave the corresponding bromide **26**[22] or iodide **27**, respectively (Scheme 4). Oxidative demethylation of these compounds with cerium(IV) ammonium nitrate in aqueous acetonitrile afforded the quinoline-5,8-diones **28**[13b] and **29**. Treatment of **28** or **29** with 3-methyl-1-dimethylamino-1-azadiene in $ClCH_2CH_2Cl$ at 80 °C afforded the fully aromatic derivatives **30** and **31**, respectively. The reaction of bromoquinoline-5,10-dione **30** with (*N*,*N*-dimethylamino)trimethyltin in toluene in the presence of 5 mol% of $PdCl_2(CN)_2$[23] afforded **32** after 6 h in 86% yield, and this compound was transformed into the hydrochloride **33** by treatment with HCl in dioxane.

The bromoquinoline-5,8-dione **34** was obtained (Scheme 5) as previously described.[24] A hetero Diels–Alder reaction between **34** and crotonaldehyde dimethylhydrazone gave the 1,5-diazaanthraquinone **35**. Oxidation of **35** with CrO_3/H_2SO_4[25] gave the dicarboxylic derivative **36**. When the oxidation of **35** was carried out with SeO_2, the reaction was regioselective and gave only compound **37**, with an aldehyde group at the C-4 position.[26] Compound **37** was oxidized to the monocarboxylic derivative **38** by treatment with $NaClO_2/H_2O_2$[27] in 92% yield. An alkene derivative (compound **39**) was prepared from the aldehyde **37** by reaction with the stabilized ylide $Ph_3P{=}CHCN$, which afforded a 10:1 mixture of *Z* and *E* isomers but only the *Z* isomer was isolated as a pure compound. The unusual stereochemical outcome of the Wittig reaction may be ascribed to steric compression by the neighbouring $C_{10}{=}O$ group. Finally, compounds **40** and **41** (Fig. 3) were obtained as previously described.[28]

2.2. Antiproliferative activity

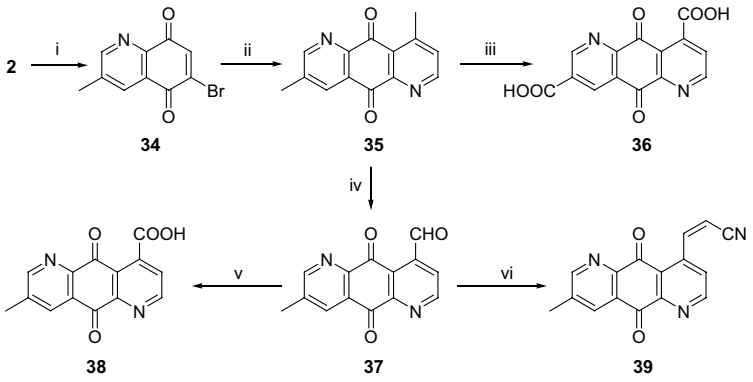
The antiproliferative activity of the 1,5-diazaanthraquinone compounds was evaluated using a panel of five human cell lines. The results are shown in Tables 1 and 2. Data for mitoxantrone are included for comparison.

The IC_{50} values (the drug concentration inhibiting the growth value of cell lines by 50%) show that the



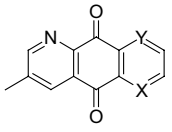
25 | 26 R=Br, 27 R=I | 28 R=Br, 29 R=I | 30 R=Br, 31 R=I | 32 | 33

Scheme 4. Reagents and conditions: (i) LiBr or LiI, 1,4-dioxane, reflux, 96 h; (ii) $(NH_4)_2Ce(NO_3)_6$, CH_3CN/H_2O, rt, 2 h; (iii) $Cl_2CHCHCl_2$, 80 °C, 30 min; (iv) $PdCl_2(CN)_2$, toluene, reflux, 6 h; (v) HCl(1,4-dioxane), 1,4-dioxane, rt, 15 min.



34 | 35 | 36 | 37 | 38 | 39

Scheme 5. Reagents and conditions: (i) $ClCH_2CH_2Cl$, methacrolein dimethylhydrazone, 80 °C, 2 h; (ii) a. $CHCl_3$, Et_3N, rt, 16 h; b. 110 °C, 0.1 Torr, 2 h; (iii) CrO_3, H_2SO_4, 80 °C, 4 h; (iv) SeO_2, 1,4-dioxane, 80 °C, 24 h; (v) $NaClO_2$, H_2O_2, Na_2HPO_4, CH_3CN/H_2O, rt, 4 h; (vi) $Ph_3P{=}CHCN$, CH_2Cl_2, reflux, 1 h.



40 X=N, Y=C
41 X=C, Y=N

Figure 3.

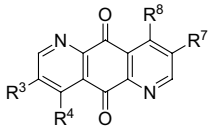
halogenated derivatives **20**, **30** and **31** were the most cytotoxic compounds, exhibiting an activity about 30-fold higher than mitoxantrone for the cell lines A549, (lung), H116 (colon) and PSN1 (pancreas) and about 6-fold higher for T98G (glioblastoma), but interestingly these compounds are about 5-fold less active than mitoxantrone for SKBR3 (breast), showing that this breast cell line is substantially less sensitive to these 1,5-diazaanthraquinone derivatives than to mitoxantrone. Activity for these three halogenated derivatives is almost identical, showing that the nature of the halogen (Cl for **20**, Br for **30** and I for **31**) is unimportant for the activity. When instead of the halogen atom an electron-donating group such as dimethylamino or methyl is present, such as in the case of derivatives **32**, **33** and **35**, respectively, a clear loss of activity is observed.

When one or both methyl groups in **35** are transformed into a carboxylic acid group (compounds **38** and **36**, respectively) a total loss of the activity was observed. On the other hand, when the C-8 methyl group of **35** was transformed into an aldehyde function (compound **37**), an increase in the cytotoxic activity was observed. However, the transformation of the aldehyde group in **37** into the conjugated nitrile, present in derivative **39**, was accompanied by a slight decrease in the activity. The activities of the 1,5-diazaanthraquinone **40** and its 1,8-diaza analogue **41** were similar in the cell lines assayed.

None of the quinoline-5,8-dione derivatives showed significant activity (Table 2), showing that the full 1,5-diazaanthraquinone chromophore is necessary for having high cytotoxic activity.

Cytotoxic activities for compounds **4a–e** and **22–24** were previously reported against a different panel of cell lines but they have not been re-evaluated in the panel used in this work.[15]

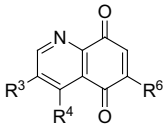
Table 1. In vitro cytotoxic activity (IC_{50}, μM) of 1,5-diazaanthraquinone derivatives against human cancer cell lines[a]



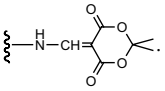
Compound	R^3	R^4	R^7	R^8	A549	H116	PSN1	T98G	SKBR3
16	H	OH	H	Phenyl	14.49	7.73	5.96	11.26	7.45
17	H	OMe	H	Phenyl	10.55	10.55	4.74	>16	15.82
18	H	OMe	H	2-Nitrophenyl	>14	>14	>14	>14	>14
20	H	Cl	Me	H	0.32	0.39	0.26	1.93	3.86
21	H	Cl	H	2-Nitrophenyl	13.68	6.39	9.12	>14	10.26
30	H	Br	Me	H	0.18	0.12	0.12	1.21	3.30
31	H	I	Me	H	0.14	0.15	0.14	1.05	2.38
32	H	NMe_2	Me	H	3.12	9.36	15.61	7.49	12.48
33	H	NMe_2·HCl	Me	H	3.29	6.58	12.07	8.23	13.72
35	Me	H	H	Me	>21	14.00	21.00	6.30	21.01
36	COOH	H	H	COOH	>17	>17	>17	>17	>17
37	Me	H	H	CHO	0.33	0.20	5.95	7.94	2.64
38	Me	H	H	COOH	>19	>19	>19	>19	>19
39	Me	H	H	CH=CH–CN	7.88	1.82	3.64	9.27	10.30
40	Me	H	H	H	3.72	2.97	8.18	8.92	14.87
41					2.23	2.23	2.97	2.23	11.16
Mitoxantrone					7.25	6.44	4.51	8.06	0.70

[a] A549, human lung carcinoma; H116, human colon carcinoma; PSN1, human pancreas adenocarcinoma; T98G, caucasian human glioblastoma; SKBR3, human breast carcinoma. All values are the mean of at least three experiments.

Table 2. In vitro cytotoxic activity (IC_{50}, μM) of quinoline-5,8-dione derivatives against human cancer cell lines[a]



Compound	R^3	R^4	R^6	A549	H116	PSN1	T98G	SKBR3
11	H	Phenyl	H	14.19	8.51	10.64	21.28	10.64
12	H	2-Nitrophenyl	NH_2	3.95	8.47	14.12	4.52	12.71
13	H	Phenyl	NH_2	18.00	9.67	14.00	17.50	15.00
14	H	2-Nitrophenyl	A	>11	>11	>11	>11	>11
15	H	Phenyl	A	>12	>12	>12	>12	>12
28	H	Br	Br	5.56	2.12	4.77	5.56	3.71
29	H	I	Br	13.78	13.78	10.34	>14	13.78
34	Me	H	Br	3.97	1.98	1.98	2.64	9.92

A represents:  .

[a] A549, human lung carcinoma; H116, human colon carcinoma; PSN1, human pancreas adenocarcinoma; T98G, caucasian human glioblastoma; SKBR3, human breast carcinoma. All values are the mean of at least three experiments.

3. Conclusion

In conclusion, a series of 1,5-diazaanthraquinone derivatives have been synthesized and their in vitro cytotoxic activities were evaluated against several human cancer cell lines. The 1,5-diazaanthraquinone chromophore has been synthesized either on the basis of hetero Diels–Alder reactions involving different quinoline-5,8-diones and α,β-unsaturated aldehyde *N*,*N*-dimethylhydrazones or by thermolysis of different arylaminomethylene Meldrum's acid derivatives. Some of these compounds showed cytotoxic activity higher than mitoxantrone. Some structure–activity relationships have been described. Further studies on this interesting class of antitumour compounds are in progress in our laboratories.

4. Experimental

The reagents used were of commercial origin (Aldrich, Fluka) and were employed without further purification. Solvents (SDS, Scharlau) were purified and dried by standard procedures. Reactions were monitored by

thin-layer chromatography, using Macherey-Nagel or Merck plates with fluorescent indicator. Separations by flash liquid chromatography were performed using silica gel from SDS 60 ACC (230–400 mesh) or Merck (60, 40–63 μm).

Melting points are uncorrected, and were determined using a Hoffler hot stage microscope. Spectroscopic data were obtained with the following instruments: IR, Perkin Elmer Paragon 1000 FT-IR; NMR, Bruker AC-250 (250 MHz for ^{1}H and 63 MHz for ^{13}C), Varian Unity 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) or Varian Unity Inova 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C). Combustion elemental analyses were obtained by the Servicio de Microanálisis Elemental, Universidad Complutense, using Perkin Elmer 2400 CHN and Leco CHNS 932 microanalyzers.

4.1. Double hetero Diels–Alder reactions for the synthesis of symmetrically substituted 1,5-diazaanthraquinones. General procedure

To a solution of 2,5-dibromobenzoquinone[29] (100–200 mg, 0.375–0.750 mmol) in chloroform (10–15 mL) was added the suitable azadiene (2 equiv), and in the case of 4-substituted azadienes, triethylamine (2 equiv). After stirring at room temperature for 1 min, the solution was evaporated. In the reactions using triethylamine, the residue was washed with H_2O. In the other reactions, the residue was washed with ethyl ether, affording the desired 1,5-diazaanthraquinones.

4.1.1. 3,8-Diethylpyrido[2,3-*g*]quinoline-5,10-dione (4a, R^3 = Et, R^4 = H). Yield, 68%. Found: C, 71.86; H, 5.50; N, 10.31. $C_{16}H_{14}N_2O_2$ requires: C, 72.18; H, 5.26; N, 10.53; mp 218–220 °C; υ (KBr): 1682.6 (C=O) cm^{-1}. δ_H ($CDCl_3$, 300 MHz): 8.93 (d, 2H, J = 2.1 Hz), 8.50 (d, 2H, J = 2.1 Hz), 2,85 (q, 4H, J = 7.5 Hz), 1.35 (t, 6H, J = 7.5 Hz); δ_C ($CDCl_3$, 75 MHz): 181.65, 155.68, 146.50, 145.57, 135.54, 130.58, 26.56, 14.83; MS (APCI-positive) *m/z*: 267 $[M+H]^+$.

4.1.2. 3,8-Dimethylpyrido[2,3-*g*]quinoline-5,10-dione (4b, R^3 = Me, R^4 = H). Yield, 89%. Found: C, 69.70; H, 4.59; N, 11.54. $C_{14}H_{10}N_2O_2$ requires: C, 70.58; H, 4.20; N, 11.76; mp > 300 °C; υ (KBr): 1682.6 (C=O) cm^{-1}. δ_H ($CDCl_3$, 300 MHz): 8.95 (d, 2H, J = 1.9 Hz), 8.51 (d, 2H, J = 1.9 Hz), 2.58 (s, 6H); δ_C ($CDCl_3$, 75 MHz): 181.42, 156.07, 146.15, 139.55, 135.54, 130.22, 18.95; MS (APCI-positive) *m/z*: 239 $[M+H]^+$.

4.1.3. 4,9-Dimethylpyrido[2,3-*g*]quinoline-5,10-dione (4c, R^3 = H, R^4 = Me). Yield, 88%. Found: C, 69.16; H, 4.49; N, 11.43. $C_{14}H_{10}N_2O_2$ requires: C, 70.58; H, 4.20; N, 11.76; mp 253–254 °C; υ (KBr): 1682.5 (C=O) cm^{-1}. δ_H ($CDCl_3$, 300 MHz): 8.85 (d, 2H, J = 5.1 Hz), 7.3 (d, 2H, J = 5.1 Hz), 2.53 (s, 6H); δ_C ($CDCl_3$, 75 MHz): 185.03, 154.23, 150.10, 145.71, 134.21, 128.17, 21.08; MS (APCI-positive) *m/z*: 239 $[M+H]^+$.

4.1.4. 9-Ethyl-3-methylpyrido[2,3-*g*]quinoline-5,10-dione (4d, R^3 = Me, R^4 = Et). Yield, 70%. Found: C, 67.71; H, 3.15; N, 13.08. $C_{12}H_6N_2O_2$ requires: C, 68.57; H, 2.88; N, 13.33; mp 180–182 °C; υ (KBr): 1680.0 (C=O) cm^{-1}. δ_H ($CDCl_3$, 300 MHz): 8.81 (s, 2H), 3.19 (q, 4H, J = 7.9 Hz), 2.53 (s, 6H), 1.31 (t, 6H, J = 7.9 Hz); δ_C ($CDCl_3$, 75 MHz): 181.3, 156.01, 152.78, 141.9, 137.60, 129.30, 23.08, 16.30, 14.21; MS (ESI-positive) *m/z*: 295 $[M+H]^+$.

4.1.5. Pyrido[2,3-*g*]quinoline-5,10-dione (4e, R^3 = R^4 = H). Yield, 79%. Found: C, 69.70; H, 4.59; N, 11.54. $C_{14}H_{10}N_2O_2$ requires: C, 70.58; H, 4.20; N, 11.76; mp > 300 °C; υ (KBr): 1681.3 (C=O) cm^{-1}. δ_H ($CDCl_3$, 300 MHz): 9.03 (dd, 2H, J = 4.8 and 2.0 Hz), 8.51 (dd, 2H, J = 8.2 and 2.0 Hz), 7.59 (dd, 2H, J = 8.2 and 4.8 Hz,); δ_C ($CDCl_3$, 75 MHz): 183.91, 151.03, 144.07, 124.2, 129.14, 125.20.

4.1.6. 5,8-Dimethoxy-4-(2-nitrophenyl)quinoline (8). A mixture of **7** (1.99 g, 5.88 mmol), 2-nitrophenyltrimethylstannane (2.86 g, 10 mmol), $Pd(PPh_3)_4$ (580 mg, 0.5 mmol), CuBr (422 mg, 2.9 mmol), in 1,4-dioxane (64 mL) was heated at 90 °C, under argon for 90 min. After cooling to room temperature, the reaction mixture was diluted with AcOEt, washed with H_2O and the organic layer dried over Na_2SO_4. The resulting crude was chromatographed (AcOEt) to afford **8** (1.53 g, 84%). Found: C, 66.05; H, 4.56; N, 9.06. $C_{17}H_{14}N_2O_4$ requires: C, 65.80; H, 4.55; N, 9.03; δ_H ($CDCl_3$, 300 MHz): 8.96 (d, 1H, J = 4.1 Hz), 8.17 (dd, 1H, J = 1.5 and 8.0 Hz), 7.54 (m, 1H), 7.45 (m, 1H), 7.30 (dd, 1H, J = 1.5 and 8.0 Hz), 7.20 (d, 1H, J = 4.1 Hz), 6.96 (d, 1H, J = 8.5 Hz), 6.68 (d, 1H, J = 8.5 Hz), 4.06 (s, 3H) 3.39 (s, 3H); δ_C ($CDCl_3$, 75 MHz): 149.92, 148.81, 144.21, 140.74, 132.48, 132.10, 131.96, 131.87, 130.93, 128.53, 128.37, 128.08, 123.41, 107.15, 105.40, 56.13, 55.52.

4.1.7. 4-(2-Nitrophenyl)quinoline-5,8-dione (10). To a mixture of **8** (1.53 g, 4.96 mmol) and CH_3CN (28 mL) was added a solution of ammonium cerium(IV) nitrate (11.16 g, 20.25 mmol) in H_2SO_4 (2 M) (41 mL). After stirring for 3 h, the reaction mixture was partitioned between AcOEt and saturated NH_4Cl aqueous solution. The organic layer was dried over Na_2SO_4, and evaporation of the solvent gave compound **10** (720 mg, 52%). Found: C, 64.19; H, 2.90; N, 10.03. $C_{15}H_8NO_4$ requires: C, 64.29; H, 2.88; N, 10.00; δ_H ($CDCl_3$, 300 MHz): 9.08 (d, 1H, J = 4.9 Hz), 8.32 (dd, 1H, J = 1.3 and 7.8 Hz), 7.75 (td, 1H, J = 1.3 and 7.8 Hz), 7.67 (td, 1H, J = 1.5 and 7.8 Hz), 7.44 (d, 1H, J = 4.9 Hz), 7.22 (dd, 1H, J = 1.5 and 7.8 Hz), 7.14 (d, 1H, J = 10.5 Hz), 6.87 (d, 1H, J = 10.5 Hz); δ_C ($CDCl_3$, 75 MHz): 184.71, 182.74, 153.72, 148.63, 147.52, 138.82, 137.73, 133.87, 132.11, 131.87, 131.74, 129.79, 129.13, 128.55, 124.55.

4.1.8. 6-Amino-4-(2-nitrophenyl)quinoline-5,8-dione (12). To a degassed solution of **10** (720 mg, 2.57 mmol) in methanol (42 mL) was added NaN_3 (1 g, 15.4 mmol) in 6.8 mL of degassed H_2O. Then, HCl (1 M, 10 mL) was added to pH = 4, and the mixture was stirred for 3 h 30 min. The solution was diluted with H_2O and ex-

tracted with AcOEt. The organic layers were dried over Na_2SO_4 and the resulting crude was chromatographed (CH_2Cl_2/MeOH; 250:5 → 100:3 → 100:10) to afford **12** (556 mg, 73%). Found: C, 61.16; H, 3.08; N, 14.28. $C_{15}H_9N_3O_4$ requires: C, 61.02; H, 3.07; N, 14.23; υ (KBr): 1686, 1604, 1569, 1525 cm^{-1}. δ_H ($CDCl_3$, 300 MHz): 8.92 (d, 1H, J = 4.9 Hz), 8.27 (dd, 1H, J = 1.2 and 8.3 Hz), 7.74 (td, 1H, J = 1.2 and 7.4 Hz), 7.65 (ddd, 1H, J=1.6, 7.4 and 8.3 Hz), 7.34 (d, 1H, J = 4.9 Hz), 7.23 (dd, 1H, J = 1.6 and 7.4 Hz), 6.07 (s, 1H); δ_C ($CDCl_3$, 75 MHz): 189.04, 181.91, 154.01, 150.49, 149.97, 148.9, 147.17, 134.70, 134.41, 130.36, 129.84, 127.62, 124.91, 124.55, 103.37; MS (ESI-negative) m/z: 294 $[M-H]^-$.

4.1.9. 6-Amino-4-phenylquinoline-5,8-dione (13). Same procedure as for **12** involving **11**[19] (295 mg, 1.25 mmol). The reaction mixture was stirred for 4 h. The crude was chromatographed (CH_2Cl_2/MeOH; 100:5) to afford **13** (200 mg, 64%). Found: C, 71.99; H, 4.02; N, 11.18. $C_{15}H_{10}N_2O_2$ requires: C, 71.99; H, 4.03; N, 11.19; mp 276–279 °C; δ_H ($CDCl_3$ + methanol-d_4, 500 MHz): 8.84 (d, 1H, J = 4.9 Hz), 7.41 (m, 3H), 7.33 (d, 1H, J = 4.9 Hz), 7.21 (m, 2H), 6.06 (s, 1H); δ_C ($CDCl_3$ + methanol-d_4, 125 MHz): 181.73, 181.11, 153.07, 151.64, 150.20, 149.99, 138.32, 129.08, 128.38, 128.11 (2C), 127.52 (2C), 124.49, 103.06; MS (ESI-negative) m/z: 249 $[M-H]^-$.

4.1.10. 6-[(2,2-Dimethyl-4,6-dioxo-[1,3]dioxan-5-ylidenemethyl)amino]-4-(2-nitrophenyl)quinoline-5,8-dione (14). A solution of Meldrum's acid (318 mg, 22 mmol) and triethylorthoformate (6.4 mL) was refluxed for 2 h under argon. The reaction mixture was added to **12** (480 mg, 1.62 mmol) and refluxed for 4 h. After being cooled to 0 °C, the solution was filtered and washed with cold methanol to yield **14** (615 mg, 84%). Found: C, 58.91; H, 3.22; N, 9.28. $C_{22}H_{15}N_3O_8$ requires: C, 58.80; H, 3.36; N, 9.35; δ_H ($CDCl_3$, 300 MHz): 9.10 (d, 1H, J = 4.9 Hz), 8.47 (d, 1H, J = 13.9 Hz), 8.35 (d, 1H, J = 7.9 Hz), 7.77 (t, 1H, J = 7.9 Hz), 7.70 (td, 1H, J = 1.4 and 7.9 Hz), 7.43 (d, 1H, J = 4.9 Hz), 7.21 (dd, 1H, J = 1.4 and 7.9 Hz), 6.91 (s, 1H), 1.73 (s, 3H), 1.72 (s, 3H); δ_C ($CDCl_3$, 75 MHz): 181.01, 179.16, 163.93, 161.98, 154.95, 149.39, 148.19, 148.08, 146.46, 141.12, 134.19, 133.72, 129.90, 129.72, 128.08, 125.15, 124.37, 113.14, 105.92, 94.44, 27.34.

4.1.11. 6-[(2,2-Dimethyl-4,6-dioxo-[1,3]dioxan-5-ylidenemethyl)amino]-4-phenylquinoline-5,8-dione (15). The same procedure as for **14** involving **13** (200 mg, 0.8 mmol) was used to yield **15** (245 mg, 76%). Found: C, 65.39; H, 3.92; N, 7.02. $C_{22}H_{16}N_2O_6$ requires: C, 65.34; H, 3.99; N, 6.93; mp 257.8–259.4 °C; δ_H ($CDCl_3$ + methanol-d_4, 500 MHz): 8.91 (d, 1H, J = 4.9 Hz), 8.51 (s, 1H), 7.45 (d, 1H, J = 4.9 Hz), 7.39 (m, 3H), 7.19 (m, 2H), 6.93 (s, 1H), 1.65 (s, 6H); δ_C ($CDCl_3$ + methanol-d_4, 125 MHz): 181.36, 178.62, 163.80, 162.46, 153.83, 152.80, 148.71, 148.50, 141.54, 137.40, 130.59, 128.84, 128.34 (2C), 127.60 (2C), 124.43, 112.92, 105.90, 93.95, 27.10 (2C).

4.1.12. 4-Hydroxy-9-phenylpyrido[2,3-*g*]quinoline-5,10-dione (16). A solution of **15** (230 mg, 0.57 mmol) in degassed diphenyl ether (36 mL), was heated between 200 and 220 °C for 15 min. The reaction mixture was cooled to room temperature and added to hexane at 0 °C. Filtration of the reaction afforded **16** (172 mg, 100%) as a brown solid. Found: C, 71.43; H, 3.35; N, 9.29. $C_{18}H_{10}N_2O_3$ requires: C, 71.52; H, 3.33; N, 9.27; mp > 280 °C; υ (KBr): 1695 (C=O), 1676 (C=O) cm^{-1}. δ_H (trifluoroacetic acid-d_4 500 MHz): 9.28 (d, 1H, J = 5.6 Hz), 8.90 (d, 1H, J = 6.6 Hz), 8.42 (d, 1H, J = 5.6 Hz), 7.82 (d, 1H, J = 6.6 Hz), 7.68 (t, 1H, J = 7.3 Hz), 7.58 (dd, 2H, J = 7.3, 7.8 Hz), 7.46 (d, 2H, J = 7.8 Hz); δ_C (trifluoroacetic acid-d_4 500 MHz): 177.89, 174.23, 171.65, 165.54, 147.29, 146.75, 142.88, 141.44, 135.79, 133.62, 132.95, 129.49 (2C), 128.38 (2C), 127.01, 121.15, 114.62; MS (ESI-negative) m/z: 301 $[M-H]^-$.

4.1.13. 4-Methoxy-9-phenylpyrido[2,3-*g*]quinoline-5,10-dione (17). To a solution of **16** (20 mg, 0.066 mmol) in 3 mL of CH_2Cl_2/MeOH (1:1) was added 20 mL of a solution of diazomethane in ethyl ether. After 10 min, the resulting mixture was evaporated to dryness and the crude was purified under flash chromatography (CH_2Cl_2/MeOH; 95:5) to afford **17** (4 mg, 19%). Found: C, 72.21; H, 3.83; N, 8.84. $C_{19}H_{12}N_2O_3$ requires: C, 72.15; H, 3.82; N, 8.86; δ_H ($CDCl_3$, 500 MHz): 9.06 (d, 1H, J = 4.9 Hz), 8.85 (d, 1H, J = 5.8 Hz), 7.54 (d, 1H, J = 4.9 Hz), 7.46 (m, 3H), 7.31 (m, 2H), 7.20 (d, 1H, J = 5.8 Hz), 4.13 (s, 3H); δ_C ($CDCl_3$, 125 MHz): 182.03, 180.24, 166.29, 155.83, 154.00, 152.40, 151.71, 150.30, 137.98, 130.06, 128.58, 128.41 (2C), 128.05 (2C), 127.33, 119.53, 111.05, 56.98; MS (ESI-positive) m/z: 317 $[M+H]^+$, 339 $[M+Na]^+$.

4.1.14. 4-Methoxy-9-(2′-nitrophenyl)pyrido[2,3-*g*]quinoline-5,10-dione (18). A solution of **14** (100 mg, 0.22 mmol) in degassed diphenyl ether (14 mL), was heated between 200 and 220 °C for 15 min. The reaction mixture was cooled to room temperature and added to hexane at 0 °C. Filtration of the reaction afforded a residue that was used without purification. To a solution of the former residue in 10 mL of CH_2Cl_2/MeOH (1:1) was added 50 mL of a solution of diazomethane in ethyl ether. After 10 min, the resulting mixture was evaporated to dryness and the crude was purified under flash chromatography (CH_2Cl_2/MeOH; 95:5) to afford the known **18**[13b] (14 mg, 17%). Spectral data for this compound were identical to those previously described.

4.1.15. 9-Chloro-3-methylpyrido[2,3-*g*]quinoline-5,10-dione (20). To a solution of quinone **19**[13c] (318 mg, 1.17 mmol) in CH_3CN (10 mL) was added a solution of methacrolein dimethylhydrazone (224 mg, 2 mmol) in ethyl ether (2 mL). The violet solution was stirred at room temperature for 16 h and evaporated to dryness. The residue was chromatographed on silica gel (AcOEt/CH_2Cl_2; 4:1) to yield **20** (241 mg, 80%) as a brown pale solid. Found: C, 60.20; H, 2.58; N, 10.99. $C_{13}H_7ClN_2O_2$ requires: 60.38; H, 2.71; N, 10.83; mp 208–210 °C; υ (KBr): 1691 (C=O) cm^{-1}; δ_H ($CDCl_3$, 250 MHz): 8.93 (d, 1H, J = 2 Hz), 8.90 (d, 1H, J = 5.1 Hz), 8.43 (d, 1H, J = 2 Hz), 7.75 (d, 1H, J = 5.1 Hz), 2.55 (s, 3H); δ_C ($CDCl_3$, 63 MHz): 180.88, 179.59, 156.97, 154.06, 150.53, 146.71, 146.50, 139.70,

135.35, 131.53, 129.02, 127.88, 19.13; MS (ESI-positive) 259 $[M+H]^+$, 281 $[M+H]^+$.

4.1.16. 4-Chloro-9-(2-nitrophenyl)pyrido[2,3-*g*]quinoline-5,10-dione (21). A solution of quinone **19**[13c] (185 mg, 0.68 mmol) and *o*-nitrocinnammaldehyde dimethylhydrazone (438 mg, 2 mmol) in chloroform (1 mL) was sonicated at 50 °C for 125 h in an ultrasound cleaning bath. The solvent was evaporated and the residue was chromatographed on silica gel (AcOEt) to afford the known compound **21**[13b,14] (52 mg, 20%). Found: C, 59.89; H, 1.99; N, 11.17. $C_{18}H_8ClN_3O_4$ requires: 59.13; H, 2.18; N, 11.48.

4.1.17. 9-Chloro-1-dimethylamino-4-methyl-1,4-dihydropyrido[2,3-*g*]quinoline-5,10-dione (22). To a solution of quinone **19**[13c] (77 mg, 0.29 mmol) in CH_3CN (5 mL) was added a solution of crotonaldehyde dimethylhydrazone (52 mg, 0.46 mmol) in ethyl ether (1 mL). The violet solution was stirred at room temperature for 22 h. The solvent and excess azadiene were evaporated under reduced pressure, and the residue was chromatographed on silica gel (AcOEt) to afford **22** (76 mg, 89%). Found: C, 59.81; H, 4.23; N, 14.02. $C_{15}H_{14}ClN_3O_2$ requires: 59.34; H, 4.61; N, 13.83; υ (KBr): 1667, 1640 (C=O) cm^{-1}; δ_H ($CDCl_3$, 250 MHz): 8.73 (d, 1H, $J = 5.3$ Hz), 7.53 (d, 1H, $J = 5.3$ Hz), 6.25 (d, 1H, $J = 7.9$ Hz), 5.20 (dd, 1H, $J = 7.9$ and 5.1 Hz), 3.76 (m, 1H), 2.69 (s, 6H), 1.17 (d, 3H, $J = 6.6$ Hz); δ_C ($CDCl_3$, 63 MHz): 180.20, 177.92, 152.74, 152.39, 149.48, 146.37, 143.09, 128.75, 121.46, 120.30, 120.16, 113.34, 44.85, 26.04, 23.95.

4.1.18. 4-Chloro-9-methylpyrido[2,3-*g*]quinoline-5,10-dione (23). A sample of compound **22** (43 mg, 0.14 mmol) was heated at 110 °C and 0.1 Torr during 2 h and then washed with ethyl ether and chloroform. The residue obtained (22 mg, 60%) was identified as compound **23**. Found: C, 60.79; H, 2.23; N, 11.11. $C_{13}H_7ClN_2O_2$ requires: C, 60.38; H, 2.71; N, 10.83; mp > 300 °C; υ (KBr): 1689 (C=O) cm^{-1}; δ_H (DMSO-d_6, 250 MHz,): 8.92 (d, 1H, $J = 5.1$ Hz), 8.87 (d, 1H, $J = 4.8$ Hz), 7.97 (d, 1H, $J = 5.1$ Hz), 7.70 (d, 1H, $J = 4.8$ Hz), 2.77 (s, 3H).

4.1.19. 4-Chloro-9-dimethylaminopyrido[2,3-*g*]quinoline-5,10-dione (24). A solution of compound **22** (21 mg, 0.11 mmol) in THF (2 mL) and aqueous HCl (6 M) (2 mL) was heated at 80 °C for 1 h. The reaction mixture was saturated with solid sodium carbonate and extracted with $CHCl_3$ and AcOEt. The combined organic layers were dried over Na_2SO_4 and evaporated, yielding **24** (16 mg, 88%). Found: C, 67.52; H, 4.48; N, 15.43. $C_{15}H_{13}N_3O_2$: 67.40; H, 4.90; N, 15.72; mp 97–100 °C; υ (KBr): 1683 and 1654 (C=O) cm^{-1}; δ_H ($CDCl_3$, 250 MHz): 8.84 (d, 1H, $J = 4.9$ Hz), 8.53 (d, 1H, $J = 5.1$ Hz), 7.44 (d, 1H, $J = 4.9$ Hz), 6.99 (d, 1H, $J = 6.1$ Hz), 3.11 (s, 6H), 2.86 (s, 3H).

4.1.20. 6-Bromo-4-iodo-5,8-dimethoxyquinoline (27). A suspension of **25**[22] (1.5 g, 3.6 mmol) and lithium iodide (1.4 g, 10.8 mmol) in 1,4-dioxane (150 mL) was refluxed for 96 h. The mixture was cooled to room temperature and diluted with AcOEt (250 mL), washed with H_2O. The aqueous phase was extracted to AcOEt. The combined organic phases were dried over Na_2SO_4 and evaporated. The resulting crude was purified by silica gel column chromatography (hexane/AcOEt; 1:1) affording **27** (931 mg, 66%) as a beige solid. Found: C, 33.64; H, 2.33; N, 3.56. $C_{11}H_9BrINO_2$ requires: C, 33.53; H, 2.30; N, 3.55; δ_H ($CDCl_3$, 300 MHz): 8.32 (d, 2H, $J = 4.8$ Hz), 8.14 (d, 2H, $J = 4.8$ Hz), 7.16 (s, 1H), 4.01 (s, 3H), 3.81 (s, 3H); δ_C ($CDCl_3$, 75 MHz): 152.31, 148.40, 145.30, 140.45, 137.68, 125.21, 116.18, 112.81, 98.46, 62.65, 56.67; MS (ESI positive) *m/z*: 396 $[M+2H]^+$.

4.1.21. 6-Bromo-4-iodoquinoline-5,8-dione (29). To a solution of **27** (250 mg, 0.63 mmol) in AcCN/H_2O (42:10 mL) at 0 °C, a solution of ammonium cerium(IV) nitrate (1.74 g, 3.17 mmol) in H_2O (2.2 mL) was dropwise added. The mixture was stirred at room temperature for 2 h and partitioned between H_2O and CH_2Cl_2. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated. The crude was triturated with ethyl ether affording **29** (230 mg, 98%) as orange solid. Found: C, 29.78; H, 0.85; N, 3.86. $C_9H_3BrINO_2$ requires: C, 29.70; H, 0.83; N, 3.85; mp 182.3–185.0 °C; δ_H ($CDCl_3$, 300 MHz): 8.38 (d, 1H, $J = 5.0$ Hz), 8.34 (d, 1H, $J = 5.0$ Hz), 7.66 (s, 1H); δ_C ($CDCl_3$, 75 MHz): 179.15, 176.00, 152.82, 148.33, 141.85, 140.41, 139.32, 128.20, 106.27; MS (APCI-positive) *m/z*: 364 $[M+H]^+$.

4.1.22. 9-Bromo-3-methylpyrido[2,3-*g*]quinoline-5,10-dione (30). To a solution of **28**[14] (500 mg, 1.57 mmol) in 1,2-dichloroethane (13 mL) at 80 °C, methacrolein dimethylhydrazone (235 mg, 2.01 mmol) in 1,2-dichloroethane (4 mL) was dropwise added. The mixture was kept at 80 °C for 30 min, then cooled to room temperature and the solvent was evaporated. The crude was purified by silica gel column chromatography (AcOEt) affording **30** (332 mg, 68%) as a brown solid. Found: C, 51.53; H, 2.30; N, 9.27. $C_{13}H_7BrN_2O_2$ requires: C, 51.51; H, 2.33; N, 9.24; mp > 200 °C (decomp.); υ (KBr) 1687 cm^{-1}; δ_H ($CDCl_3$, 300 MHz): 8.96 (d, 1H, $J = 2.2$ Hz), 8.76 (d, 1H, $J = 5.0$ Hz), 8.45 (d, 1H, $J = 2.2$ Hz), 8.00 (d, 1H, $J = 5.0$ Hz), 2.57 (s, 3H); δ_C ($CDCl_3$, 75 MHz): 180.72, 179.71, 156.97, 153.65, 150.39, 146.58, 139.73, 135.31, 135.25, 134.61, 129.09, 129.02, 19.15; MS (APCI-positive) *m/z*: 304 $[M+H]^+$.

4.1.23. 9-Iodo-3-methylpyrido[2,3-*g*]quinoline-5,10-dione (31). Same procedure as for **30** involving **29** (50 mg, 0.13 mmol). The crude was purified by silica gel column chromatography (AcOEt) affording mainly impure fractions but only few pure **31** (7 mg, 15%) was isolated as a brown solid. Found: C, 44.78; H, 2.03; N, 8.02. $C_{13}H_7IN_2O_2$ requires: C, 44.60; H, 2.02; N, 8.00; mp > 215 °C (decomp.); υ (KBr) 1683 cm^{-1} (C=O); δ_H ($CDCl_3$, 300 MHz): 8.98 (d, 1H, $J = 2.0$ Hz), 8.57 (d, 1H, $J = 4.9$ Hz), 8.47 (d, 1H, $J = 2.0$ Hz), 8.40 (d, 1H, $J = 4.9$ Hz), 2.59 (s, 3H); δ_C ($CDCl_3$, 75 MHz): 180.37, 179.55, 156.75, 152.86, 149.37, 146.11, 142.44, 139.58, 135.08, 130.37, 129.02, 106.07, 18.94; MS (APCI-positive) *m/z*: 351 $[M+H]^+$.

4.1.24. 9-Dimethylamino-3-methylpyrido[2,3-*g*]quinoline-5,10-dione (32). To a solution of **30** (50 mg, 0.16 mmol) and $Pd(CH_3CN)_2Cl_2$ (2 mg, 0.008 mmol) in anhydrous

toluene (3.5 mL) was added dimethylaminotrimethylstannane. The mixture was refluxed for 6 h and cooled to room temperature. It was diluted with CH_2Cl_2 and washed with H_2O. The aqueous phase was extracted to CH_2Cl_2. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated. The crude was purified by silica gel column chromatography (CH_2Cl_2/MeOH; 100:1) affording **32** (37 mg, 86%) as a red solid. Found: C, 67.38; H, 4.93; N, 15.76. $C_{15}H_{13}N_3O_2$ requires: C, 67.40; H, 4.90; N, 15.72; mp 241.7–243.1 °C; δ_H ($CDCl_3$, 300 MHz): 8.88 (d, 1H, $J = 2.6$ Hz), 8.51 (d, 1H, $J = 6.1$ Hz), 8.40 (d, 1H, $J = 2.6$ Hz), 7.00 (d, 1H, $J = 6.1$ Hz), 3.10 (s, 6H), 2.53 (s, 3H); δ_C ($CDCl_3$, 75 MHz): 182.73, 180.73, 156.25, 156.17, 151.90, 151.52, 148.20, 138.44, 135.17, 128.52, 118.59, 113.33, 43.92, 18.93; MS (APCI-positive) *m/z*: 268 $[M+H]^+$.

4.1.25. Dimethyl-(8-methyl-5,10-dioxo-5,10-dihydropyrido[2,3-*g*]quinolin-4-yl)-ammonium chloride (33). To a solution of **32** (15 mg, 0.06 mmol) in the minimum amount necessary of 1,4-dioxane was added HCl (5.9 M in 1,4-dioxane) (20 μL, 0.12 mmol). The mixture was stirred at room temperature for 15 min and the resulting solid was filtered affording **33** (13 mg, 72%) as an orange solid. Found: C, 59.54; H, 4.19; N, 13.80. $C_{15}H_{14}ClN_3O_2$ requires: C, 59.31; H, 4.65; N, 13.83; mp > 270 °C (decomp.); δ_H (methanol-d_4, 300 MHz): 8.92 (br, 1H), 8.46 (br, 1H), 8.28 (d, 1H, $J = 6.6$ Hz), 7.51 (d, 1H, $J = 6.6$ Hz), 3.30 (s, 6H), 2.59 (s, 3H); δ_C (methanol-d_4, 75 MHz): 180.11, 179.14, 158.50, 157.26, 148.35, 146.37, 141.50, 141.25, 135.94, 128.92, 117.48, 114.37, 44.91, 18.66; MS (APCI-positive) *m/z*: 268 $[M-Cl]^+$, 290 $[M-Cl+Na]^+$.

4.1.26. 3,9-Dimethylpyrido[2,3-*g*]quinoline-5,10-dione (35). To a solution of **34**[24] (480 mg, 1.90 mmol) in $CHCl_3$ (9 mL), crotonaldehyde dimethylhydrazone (235 mg, 2.09 mmol) and Et_3N (260 μL, 1.90 mmol) were added. The mixture was stirred to room temperature for 16 h. The solvent was evaporated and the residue was heated at 110 °C and 0.1 Torr for 2 h. The crude was purified by silica gel column chromatography (CH_2Cl_2/AcOEt; 8:2 → 1:1) affording **35** (385 mg, 84%) as a brown solid. Found: C, 70.47; H, 4.28; N, 11.78. $C_{14}H_{10}N_2O_2$ requires: C, 70.58; H, 4.20; N, 11.76; mp 255.8–258.0 °C; δ_H ($CDCl_3$, 300 MHz): 8.92 (d, 1H, $J = 2.6$ Hz), 8.90 (d, 1H, $J = 5.3$ Hz), 8.43 (d, 1H, $J = 2.6$ Hz), 7.56 (d, 1H, $J = 5.3$ Hz), 2.94 (s, 3H), 2.59 (s, 3H); δ_C ($CDCl_3$, 300 MHz): 182.56, 181.44, 155.99, 153.25, 151.71, 149.21, 146.52, 138.80, 134.67, 131.24, 129.05, 128.73, 22.38, 18.54; MS (APCI-positive) *m/z*: 239.0 $[M+H]^+$.

4.1.27. 5,10-Dioxo-5,10-dihydropyrido[2,3-*g*]quinoline-3,9-dicarboxylic acid (36). To a solution of **35** (200 mg, 0.84 mmol) in H_2SO_4 (6 mL) was added CrO_3 (504 mg, 5.04 mmol) during 1 h in portions. The mixture was heated at 80 °C for 4 h and then stirred at room temperature overnight. The crude was diluted with H_2O and extracted to AcOEt. The combined organic phases was washed with brine, dried over Na_2SO_4 and evaporated affording **36** (100 mg, 40%) as a pale yellow solid. Found: C, 56.52; H, 2.04; N, 9.36. $C_{14}H_6N_2O_6$ requires: C, 56.39; H, 2.03; N, 9.39; δ_H (DMSO-d_6, 300 MHz): δ 9.55 (d, 1H, $J = 1.8$ Hz), 9.22 (d, 1H, $J = 4.8$ Hz), 8.95 (d, 1H, $J = 1.8$ Hz), 7.97 (d, 1H, $J = 4.8$ Hz); δ_C (DMSO-d_6, 300 MHz): 180.66, 169.15, 165.65, 155.49, 154.90, 150.88, 149.54, 143.69, 139.11, 136.13, 130.96, 130.72, 125.99, 120.00; MS (ESI-positive) *m/z*: 299 $[M+H]^+$.

4.1.28. 8-Methyl-5,10-dioxo-5,10-dihydropyrido[2,3-*g*]quinoline-4-carbaldehyde (37). To a solution of **35** (231 mg, 0.97 mmol) in 1,4-dioxane (30 mL) at 80 °C, SeO_2 (215 mg, 1.94 mmol) in 1,4-dioxane (12 mL) and H_2O (1 mL) was dropwise added. The mixture was kept at 80 °C for 8 h. The reaction was cooled to room temperature, filtered over Celite and the solvent was evaporated. The residue was again dissolved in 1,4-dioxane (30 mL), heated at 80 °C and SeO_2 (215 mg, 1.94 mmol) in 1,4-dioxane (12 mL) and H_2O (1 mL) was dropwise added. The mixture was kept at 80 °C overnight and cooled to room temperature, filtered over Celite, washed with a mixture of CH_2Cl_2/MeOH (1:1) and the solvent evaporated. The crude was purified by silica gel column chromatography (CH_2Cl_2/AcOEt/MeOH; 1:1:0.1) affording **37** (177 mg, 77%) as a brown solid. Found: C, 66.72; H, 3.19; N, 11.12. $C_{14}H_8N_2O_3$ requires: C, 66.67; H, 3.20; N, 11.11; mp > 215 °C (decomp.); δ_H ($CDCl_3$, 300 MHz): 10.84 (s, 1H), 9.26 (d, 1H, $J = 4.67$ Hz), 9.46 (d, 1H, $J = 2.19$ Hz), 8.49 (d, 1H, $J = 2.19$ Hz), 7.91 (d, 2H, $J = 4.67$ Hz), 2.59 (s, 3H); δ_C ($CDCl_3$, 300 MHz): 191.05, 182.52, 180.66, 156.76, 156.08, 149.12, 145.96, 145.61, 140.29, 135.58, 129.80, 128.88, 125.93, 19.06; MS (APCI-positive) *m/z*: 225.0 $[M-CHO+H]^+$, 253 $[M+H]^+$.

4.1.29. 8-Methyl-5,10-dioxo-5,10-dihydropyrido[2,3-*g*]quinoline-4-carboxylic acid (38). To a solution of **37** (100 mg, 0.40 mmol) in CH_3CN (4 mL), NaH_2PO_4 (12.7 mg, 0.1 mmol) in H_2O (0.5 mL), H_2O_2 (45 μL, 0.41 mmol) was added and then $NaClO_2$ (64 mg, 0.55 mmol) was dropwise added in H_2O (1 mL). The mixture was stirred at room temperature for 4 h. A catalytic amount of $Na_2S_2O_5$ and HCl (2 M) to pH = 1 was consecutively added to the reaction. The precipitated obtained was filtered affording **38** (78 mg, 73%). The mother liquors were extracted with AcOEt. The combined organic phases was washed with brine, dried over Na_2SO_4, and evaporated affording a new amount of **38** (20 mg, 19%) as a pale orange solid. Found: C, 62.80; H, 3.03; N, 10.40. $C_{14}H_8N_2O_4$ requires: C, 62.69; H, 3.01; N, 10.44; mp > 300 °C (decomp.); δ_H (methanol-d_4, 300 MHz): 9.12 (d, 1H, $J = 4.8$ Hz), 8.94 (d, 1H, $J = 2.1$ Hz), 8.39 (d, 1H, $J = 2.1$ Hz), 7.86 (d, 1H, $J = 4.8$ Hz), 2.54 (s, 3H); δ_C (methanol-d_4, 300 MHz): 180.76, 180.42, 168.60, 155.33, 154.58, 148.87, 146.04, 143.02, 139.21, 134.50, 130.14, 126.50, 125.17, 18.27; MS (ESI-positive) *m/z*: 269 $[M+H]^+$, 559 $[2M+Na]^+$.

4.1.30. 3-(8-Methyl-5,10-dioxo-5,10-dihydropyrido[2,3-*g*]quinolin-4-yl)acrylonitrile (39). A solution of **37** (118 mg, 0.47 mmol) and (triphenylphosphoranylidene)acetonitrile (211 mg, 0.70 mmol) in anhydrous CH_2Cl_2 (118 mL) was refluxed for 1 h. The mixture

was cooled to room temperature and the solvent was evaporated. ^{1}H NMR spectra of the crude showed a 10:1 mixture of *Z*:*E* isomers. The resulting crude was purified by silica gel column chromatography (CH_2Cl_2/AcOEt/MeOH; 1:1:0.1) affording the *Z* isomer **39** (76 mg, 59%) as a pale green solid. Found: C, 69.73; H, 3.31; N, 15.30. $C_{16}H_9N_3O_2$ requires: C, 69.81; H, 3.30; N, 15.27; mp > 250 °C (decomp.); δ_H ($CDCl_3$, 300 MHz): 9.22 (d, 1H, $J = 4.9$ Hz), 8.97 (br, 1H), 8.52 (br, 1H), 8.17 (d, 1H, $J = 11.8$ Hz), 7.95 (d, 1H, $J = 4.9$ Hz), 5.88 (d, 1H, $J = 11.8$ Hz), 2.60 (s, 3H); δ_C ($CDCl_3$, 300 MHz): 182.17, 180.94, 156.64, 155.26, 149.37, 147.89, 146.11, 144.15, 139.94, 135.40, 129.22, 128.26, 126.40, 115.53, 101.31, 18.98; MS (ESI-positive) *m/z*: 276 $[M+H]^+$, 298 $[M+Na]^+$, 573 $[2M+Na]^+$. The *E* isomer was not isolated as pure compound.

4.2. Cell culture

All the tumour cell lines were obtained from the ATCC. Human lung carcinoma A549, colon adenocarcinoma H116, breast carcinoma SKBR3 and pancreatic adenocarcinoma PSN1 were cultured in RPMI medium containing glutamine (2 mM), penicillin (50 μ/mL), streptomycin (50 μg/mL), supplemented with 5% FBS (A549 and H116) or 10% FBS (SKBR3 and PSN1). Caucasian glioblastoma T98G was maintained in RPMI 1640, 2 mM glutamine, penicillin (50 IU/mL), streptomycin (50 μg/mL), 1 mM pyruvate supplemented with amino acids and 10% FBS.

4.3. Cell proliferation assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT; Sigma Chemical Co., St. Louis, MO) dye reduction assay in 96-well microplates was used, essentially as described.[30] The assay is dependent on the reduction of MTT by mitochondrial dehydrogenases of viable cell to a blue formazan product, which can be measured spectrophotometrically. Tumour cells (4×10^3 A-549 cells or 6×10^3 H-116, 6×10^3 PSN1, 6×10^3 SKBR3 and 6×10^3 T98G cells in a total volume of 200 μL of complete medium) were incubated in each well with serial dilutions (5 μg/mL, 2.5 μg/mL, 1 μg/mL, 0.5 μg/mL, 0.1 μg/mL, 0.05 μg/mL, 0.01 μg/mL, 0.005 μg/mL) of the tested compounds. After two days of incubation (37 °C, 5% CO_2 in a humid atmosphere) 50 μL of MTT (5 mg/mL in PBS) were added to each well and the plate was incubated for a further 2 h (37 °C). The resulting formazan was dissolved in 100 μL DMSO and read at 490 nm. All determinations were carried out in triplicate. IC_{50} value was calculated as the concentration of drug yielding a 50% of cell survival.

Acknowledgements

We are grateful to the Conserjería de Economía y Empleo de Castilla y León (progama ADE) for financial support of this research. S.M. is grateful to the MCyT for a predoctoral fellowship (acción MIT-Becas del Programa PACTI del plan nacional de I+D). M.J.M. is grateful to the MCyT for a postdoctoral fellowship (acción IDE-Becas del Programa del Plan Nacional de I+D). Financial support of this research through grant PTR95-0623.OP is also gratefully acknowledged. The authors thank Marta Martínez for the MS studies.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2004.09.021.

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