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New products

Aminoalkylamino derivatives of dihydroxy-benzo[g]isoquinoline dione and of trihydroxy-naphtho[2,3-g]isoquinoline dione: synthesis and anti-tumor evaluation

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Introduction

Increasing concern in the development of new drugs has focused attention on the anthraquinone series, since the discovery of anthracycline anti-tumor antibiotics (for reviews see [1, 2]). The synthesis of various anthraquinone derivatives led to the preparation of ametantrone (NSC 196473) **1a** and mitoxantrone (NSC 279836) **1b**, the latter showing outstanding anti-neoplastic activity (for a review see [3]).

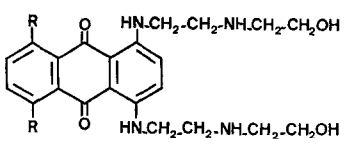
On the other hand, research carried out in this laboratory for the past 15 years has shown that the introduction of an amino alkylamino side chain into a poly condensed system, such as ellipticine, results in an increase of the anti-tumor activity, as demonstrated by the high potency of 1-(γ-diethylaminopropylamino)-5,11-dimethyl-6H-pyrido-(4,3-b]carbazole (BD84) **2a** [4, 5]. Moreover, replacement of the 9-C—OCH_3 group by a nitrogen atom enhances the activity still further [6, 7]; thus, 10-(γ-diethylamino-propylamino)-6-methyl-5H-pyrido[3′,4′-4,5]pyrrolo[2,3-g]-isoquinoline (BD40, NSC 3274718) **2b** gave positive results on numerous experimental tumors as well as against various human cancers during a phase I clinical trial [8].

These observations prompted us to investigate a series of benzo[g]isoquinoline diones bearing an aminoalkyl-amino side chain and we recently reported the synthesis of two key intermediates aimed at the preparation of nitrogen monosubstituted analogues of **1b** [9].

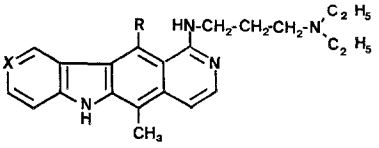
In this paper, we report the detailed synthesis of various dihydroxy-benzo[g]isoquinoline diones and of a trihydroxy-naphtho[2,3-g]isoquinoline dione as well as the preparation and the biological evaluation of some aminoalkyl-amino derivatives of these new dihydroxy polycyclic aza-quinones.

Chemistry

Lithiation of 2-(2-methoxy-4-pyridyl)-4,4-dimethyl-4,5-dihydro oxazole **3** by means of methyllithium and subsequent condensation with 2,5-dimethoxy benzaldehyde afforded oxazoline **4** [10]. Treatment of this compound in hot 6 N hydrochloric acid led to lactone **5** which resulted from oxazoline hydrolysis and concomitant demethylation of the pyridine 2-methoxy group. Moreover, lactone **5** was poorly soluble in most organic solvents and attempts



1a: R = H
1b: R = OH



2a: R = CH_3, X = C—OCH_3
2b: R = H, X = N

to oxidize it with various oxidants, including activated manganese bioxide and chromic anhydride failed to provide the corresponding ketoacid.

We also tried to oxidize oxazoline **4** with dipyridinium chlorochromate (DPCC), pyridinium chlorochromate (PCC) and pyridinium dichromate (PDC). Results were disappointing. Thus: 1) DPCC led to the recovery of the starting compound; 2) PCC oxidation afforded a mixture from which oxazolinone **6** was isolated in low yield. Moreover, this new derivative was not hydrolyzed by hot 6 N hydrochloric acid and treatment of the other compounds by boiling in the same acid gave lactone **5** corresponding to the unreacted oxazoline **4** and a small amount of ketoacid **7** arising from the keto oxazoline **8** which was not isolated; 3) PDC transformed oxazoline **4,** but acidic treatment of the resulting mixture led to ketoacid **7** in low yield.

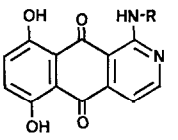
Finally, hydrolysis of oxazoline **4** in acetic acid afforded the trimethoxylactone **9** and bromination with *N*-bromosuccinimide (NBS) and subsequent treatment in boiling methanolic sodium hydroxide led to ketoacid **10** [11].

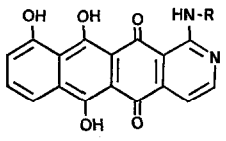
Methane sulfonic acid cyclization of this last compound, as well as that of ketoacid **7** proceeded with concomitant total *O*-demethylation, giving 6,9-dihydroxy-2H-benzo[g]-isoquinoline-1,5,10 trione **11a** in good yield (Scheme 1).

Unfortunately, attempts to substitute the hydroxyl or NH groups of compound **11a** failed and chlorination of the amidocarbonyl function by boiling in phosphorous oxychloride was inefficient.

We then chose to synthesize the corresponding chloro-derivative and the naphthacene analogue by starting from chlorooxazoline **12**. Thus, following the procedure briefly mentioned above [9], lithiation of 2-(2-chloro 4-pyridyl)-4,4-dimethyl-4,5-dihydrooxazole **12** by methyllithium and further reaction with 2,5-dimethoxy benzaldehyde afforded chlorooxazoline **13** which was hydrolyzed to lactone **14** by treatment with hot 6 N hydrochloric acid. NBS bromination followed by potassium hydroxide treatment then led to ketoacid **15** which was cyclized to 1-chloro 6,9-dihydroxy benzo[g]isoquinoline-5,10-dione **16** by methane sulfonic acid. According to the same general scheme, replacement of dimethoxy benzaldehyde by 1,4,8-trimethoxy-2-naphthaldehyde **17** gave successively: oxazoline **18,** lactone **19,** ketoacid **20** and 1-chloro-6,10,11-trihydroxy-naphtho[2,3-g]isoquinoline-5,12-dione **21** (Scheme 2).

Substitution of chloroquinones **16** and **21** was carried out using a slight excess of the required diamine (1.2 eq) in a toluene solution. Amino substituted derivatives **22a, 22b** and **23** were thus obtained in 70% yield but attempts made with a larger excess of the diamine failed, giving strongly colored material of undefined structure, as did the pure diamine.

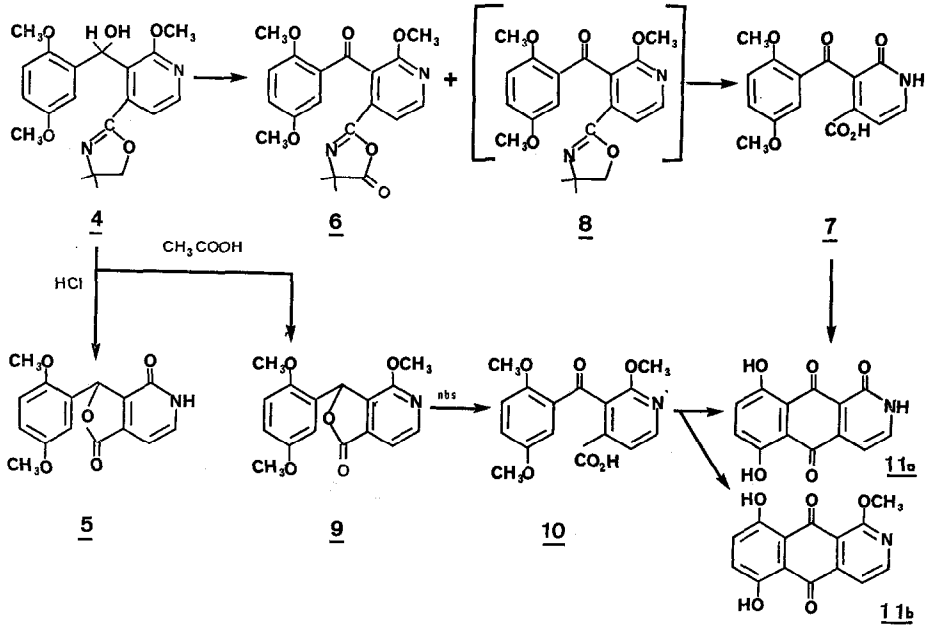




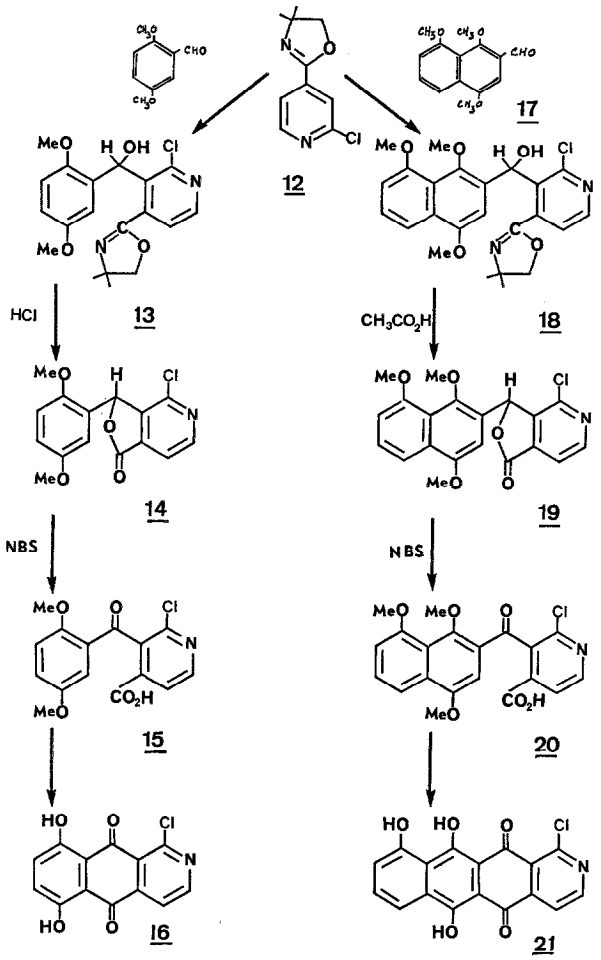
22a: R = CH_2-CH_2-CH_2-N-(C_2H_5)$_2$
22b: R = CH_2-CH_2-N-(CH_3)$_2$
22c: R = CH_2-CH_2-NH-CH_2-CH_2OH

23: R = CH_2-CH_2-CH_2-N-(C_2H_5)$_2$

However, particular mention should be made concerning substitution of chloroquinone **16** by hydroxyethylamino-ethylamine, for which the yield in the expected compound **22c** did not exceed 10%. In this case, excess of the diamine cannot be used for the above mentioned reasons, and when the reaction was performed under stoichoimetric conditions, in addition to the normal substituted derivative **22c,** a dimeric compound resulted. As demonstrated by

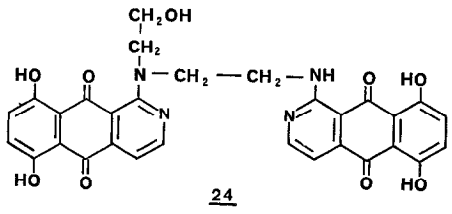


Scheme 1.



Scheme 2.

elemental analysis and mass spectra, this involves two azaanthraquinone moieties linked by a single chain. The structure of this compound has not yet been established with certainty but probably corresponds to formula **24**.



Biological evaluation and Discussion

Compounds **22** and **23** were first evaluated *in vitro* for cytostatic activity against two different murine leukemia cell lines, one of lymphocytic (L1210) and the other of erythroblastic (745) origin. Positive controls were obtained with both mitoxantrone **1b** and BD40 **2b**. Doses for a 50% growth inhibition (ID_{50}) were determined from regression curves of triplicate experiments carried out with drug concentrations ranging from 3×10^{-6} to 10^{-8} M incubated for 24 h with exponentially growing cells. Results are given in Table I.

Table I. Cytostatic activity of azaanthraquinones.

Compounds	ID_{50} (μM)	
	L1210	745
Mitoxantrone (NSC 279836)	0.003	0.004
BD40 (NSC 327471)	0.035	0.016
22a	2.2 1.6[a]	2.1
22b	0.8	1.7[b]
22c	n.d.[c]	0.8
23	n.d.[c]	3

[a]Results from two different laboratories.
[b]Theoretical value but high cytotoxic response above 1.3 μM.
[c]Not determined.

With regard to difficulties in the preparation of pure samples of **22c** and **23** and considering the poor results obtained *in vitro*, only **22a** and **22b** were studied further.

22a and **22b** affinities for calf thymus DNA *in vitro* were determined fluorometrically by competition with ethidium bromide [12] and were found to be, respectively, $3.2 \pm 1.3 \times 10^6$ M^{-1} and $5 \pm 2.6 \times 10^6$ M^{-1}. These values are comparable to the $3.6 \pm 0.6 \times 10^6$ M^{-1} value obtained with adriamycin, under the same experimental conditions.

In spite of the rather low potency of these two compounds *in vitro*, their very high affinities for DNA prompt us to evaluate their *in vivo* anti-tumor activity.

Unfortunately no significant increase of life span *(ILS)* was observed in CDF_1 mice inoculated with P388 cells at doses ranging from 10 to 40 mg/kg, whereas a 91% *ILS* was obtained with 1.5 mg/kg of adriamicyn, using the same experimental protocol.

These data suggest that the presence of a nitrogen atom in the nucleus bearing the aminoalkyl side chain dramatically changes the biological properties, in spite of a very high DNA affinity.

Such a structural modification at the other end of the molecule has been shown to have very little effect on antitumor potency [13], indicating that polarity of the aromatic nucleus is poorly related to biological properties. Thus, the presence of two aminoalkyl side chains appears to be a prerequisite for cytostatic activity. Other syntheses and biological studies are in progress in this Institute aimed at the establishment of an improved structure—activity relationship for this class of compounds.

Experimental protocols

Chemistry

All melting points were determined with a Reichert hot—stage microscope and are uncorrected. ^1H NMR spectra were recorded on a Varian XL-100 (100 MHz) spectrometer, using the FT technique (internal reference tetramethylsilane). All elemental analyses (CNRS, Paris) are within ± 0.4% of the theoretical values for the mentioned elements. IR spectra were recorded on a FTIR Nicolet 5MX spectrometer in KBr pellets.

Oxidations of 2-[2-methoxy-3-α-hydroxy-α-(2,5-dimethoxyphenyl)-pyridine-4-yl]-4,5-dihydro-4,4-dimethyloxazole ***4***

With DPCC. Treatment of **4** (1 g, 2.77 mmol), obtained as previously described [10], in dichloromethane (60 ml) with DPCC (2.1 g, 8 mmol), results in no change of the starting material, even after 4 days.

With PDC. Oxazoline **4** (1 g, 2.77 mmol) in dry dimethylformamide (DMF) (30 ml) was stirred at room temperature for 4 days with PDC (3 g, 8 mmol), then poured into 6 N hydrochloric acid and stirring was continued for 15 min. The residual mixture, obtained from extraction with dichloromethane (3 × 60 ml) and conventional treatment, was triturated with 5% aqueous sodium hydrogenocarbonate (100 ml). Insoluble material was collected by filtration and recrystallized from ethyl alcohol, to give 200 mg (26%) of **5** (mp: 236°C) [10]. The filtrate was acidified with diluted hydrochloric acid and then extracted with dichloromethane. The organic layer was washed with water, dried over sodium sulfate and evaporated under vacuum. The residue was crystallized in ethyl alcohol to afford ketoacid **7** as colorless microprisms: mp: 295°C, 145 mg (17.8%). ^1H NMR ($CDCl_3$): δ 3.56 (s, 3H, OCH_3 2′ or 5′); 3.80 (s, 3H, OCH_3 2′ or 5′); 6.51 (d, 1H, H_5); 7.06 (m, 1H, $H_{3'}$); 7.20 (m, 1H, $H_{4'}$, $J_{4',3'} = 8.9$ Hz); 7.35 (d, 1H, $H_{6'}$, $J_{4',6'} = 3$ Hz); 7.52 (d, 1H, H_6, $J_{5,6} = 6.5$ Hz). Anal. $C_{15}H_{13}NO_6$ (C, H, N).

With PCC. To a solution of **4** (1 g, 2.77 mmol) in 60 ml of dry dichloromethane, PCC (1.73 g, 8 mmol) was added. The resulting black mixture was allowed to stand for 3 days at room temperature and then poured into water and extracted with dichloromethane (3 × 50 ml). The combined organic extracts were washed several times with water, dried over sodium sulfate and then evaporated under vacuum. The organic residue was chromatographed over SiO_2 yielding two fractions.

Elution with dichloromethane gave a mixture of two compounds from which oxazoline **6** was isolated after crystallization from cyclohexane. Colorless needles, mp: 160°C, 200 mg (19%). ^1H NMR ($CDCl_3$): δ 1.23 (s, 3H, CH_3 *anti*); 1.83 (s, 3H, CH_3 *syn* with respect to C=O); 3.43 (s, 3H, OCH_3 pyridine); 3.85 (s, 3H, OCH_3); 3.88 (s, 3H, OCH_3); 6.72 (m, 1H, $H_{3'}$, $J_{3',4'} = 9$ Hz); 6.90 (m, 1H, $H_{4'}$); 7.34 (d, 1H, H_5, $J_{5,6} = 5$ Hz); 7.41 (d, 1H, $H_{6'}$, $J_{6',4'} = 2.90$ Hz); 8.40 (d, 1H, H_6). IR. ν CO = 1800 and 1732 cm^{-1}. Anal. $C_{20}H_{20}N_2O_6$ (C, H, N).

The other compound of this fraction was isolated after a second chromatography over SiO_2, affording **9**, 85 mg (10%) as small colorless needles (methyl alcohol), mp: 134°C. ^1H NMR ($CDCl_3$): δ 3.71 (s, 3H, OCH_3 5′ or 2′); 3.77 (s, 3H, OCH_3 2′ or 5′); 3.92 (s, 3H, OCH_3 pyridine); 6.48 (t, 1H, $H_{6'}$); 6.72 (d, 3H, CH_3); 6.88 (m, 2H, $H_{3'}$ + $H_{4'}$); 7.41 (d, 1H, H_7); 8.38 (q, 1H, H_6, $J_{6,7} = 5.2$ Hz; $J_{3,6} = 0.7$ Hz). Anal. $C_{16}H_{15}NO_5$ (C, H, N).

The other fraction obtained after elution with ethyl acetate was treated with boiling 6 N hydrochloric acid for 15 min and after extraction and conventional treatment the residue was washed with 10% aqueous sodium hydrogenocarbonate. Insoluble material was identified as lactone **5** (195 mg, 25%), whereas neutralization of the aqueous phase furnished ketoacid **7** after crystallization from ethyl alcohol (165 mg, 20%).

1,3-Dihydro-3-(2′,5′-dimethoxyphenyl)-4 methoxy-(3H)-furo[3,4-c]-pyridin-1-one ***9***

Oxazoline **4** (10 g, 26.8 mmol) was hydrolyzed with acetic acid (100 ml) at room temperature for 24 h, to give lactone **9** in 92% yield.

3-(2′,5′-Dimethoxybenzoyl)-2-methoxy-4-pyridine carboxylic acid ***10***

Lactone **9** (9.5 g, 31 mmol) was dissolved in hot carbon tetrachloride (700 ml) and the solution was allowed to cool at room temperature. *N*-Bromosuccinimide (7.3 g, 40 mmol) and 2,2′-azobis-2-methyl propionitrile (10 mg) were added and the resulting solution was stirred under irradiation with a mercury lamp (Mazda 125 W) for 15 min.

The reaction was gently refluxed for 6 h, allowed to cool at room temperature and then filtered. The solvent was removed under reduced pressure and the residue was treated for 30 min with 0.5 N methanolic potassium hydroxide (200 ml). The resulting solution was concentrated under vacuum, diluted with water and washed with dichloromethane (3 × 50 ml). The aqueous layer was then acidified with diluted hydrochloric acid. The precipitate was collected by filtration and treated with 5% aqueous sodium hydrogenocarbonate (100 ml) to separate the expected ketoacid **10** from unreacted starting material. After neutralization of the resulting solution, **10** was crystallized from methyl alcohol to give pale yellow crystals mp: 203°C, 5 g (50%). ^1H NMR ($CDCl_3$): δ 3.45 (s, 3H, OCH_3, pyridine); 3.86 (s, 3H, OCH_3 2′ or 5′); 3.90 (s, 3H, OCH_3 2′ or 5′); 6.84 (d, 1H, $H_{3'}$); 7.10 (d, 1H, $H_{4'}$, $J_{4',3'} = 9$ Hz); 7.43 (d, 1H, H_5); 7.60 (d, 1H, $H_{6'}$, $J_{4',6'} = 3.5$ Hz); 8.30 (d, 1H, H_6, $J_{5,6} = 5$ Hz). Anal. $C_{16}H_{15}NO_6$ (C, H, N).

Treatment of **10** with 6 N HCl overnight at room temperature furnished **7** in 90% yield.

6,9-Dihydroxy(2H)-benzo[g]isoquinoline-1,5,10-trione ***11a***

Cyclization of ketoacid 7 (1.5 g, 4.6 mmol) was carried out in methane sulfonic acid (25 ml) under nitrogen at 130°C for 3 h and at room temperature overnight. The solution was poured onto ice and the precipitate of **11a** was collected, washed several times with water and dried under vacuum. Crystallization from DMF gave deep red needles (640 mg, 53%) mp > 310°C. ^1H NMR: compound **11a** was not sufficiently soluble in conventional solvents to allow its NMR spectrum to be obtained, whereas in deuterated DMF or DMSO, only a broad envelope was obtained. IR ν CO 1701 (shoulder); 1674; 1634 cm^{-1}. Anal. $C_{13}H_7NO_5 + \frac{1}{2} H_2O$ (C, H, N).

Cyclization of trimethoxy ketoacid **10,** under the same conditions led to a mixture of **11a** and **11b,** the latter being easily separated by extraction with dichloromethane, since **11a** was almost completely insoluble in most organic solvents.

1-Methoxy-6,9-dihydroxy benzo[g]isoquinoline 5,10-dione ***11b***

Cristallized as red needles from ethyl alcohol, mp: 229°C, 10%. ^1H NMR ($CDCl_3$): δ 4.24 (s, 3H, OCH_3-1); 7.28 (d, 1H, H_7 or H_8); 7.40 (d, 1H, H_7 or H_8, $J_{7,8} = 9.4$ Hz); 7.82 (d, 1H, H_4); 8.67 (d, 1H, H3, $J_{3,4} = 5.3$ Hz); 12.65 (s, 1H6 or OH9); 13.16 (s, 1H, OH9 or OH6).

1,3-Dihydro-3-(2,5-dimethoxyphenyl)-4-chloro(3H)pyrido[3,4-c]furan-1-one ***14***

Lithiation of 2-(2-chloro-4-pyridyl)-4,4-dimethyl-4,5-dihydro oxazole **12** [9] (3.2 g, 15 mmol) with methyl lithium (20 ml of a 0.9 N ether solution, 1.2 eq) in dry THF (60 ml) under nitrogen at —70°C led to a yellow solution. After 1 h at this temperature, dimethoxy benzaldehyde (2.6 g, 15 mmol) in THF (20 ml) was added and the mixture was allowed to stand overnight at room temperature. The solution was poured into water and the suspension extracted with toluene (3 × 100 ml). The combined extracts were washed with water, dried over sodium sulfate and evaporated under vacuum. The brown residue was triturated with toluene (10 ml) until crystallization and the colorless crystals of **13** were collected by filtration (4.1 g, 72%).

Attempts to further purify this raw product led to a partial opening of the oxazole ring as shown by NMR spectroscopy with the appearance of an OH signal highfield with a triplet configuration characteristic of a CH_2OH group.

The raw product (10 g, 26 mmol) was refluxed in 6 N hydrochloric acid (200 ml) overnight. The crystalline product formed by cooling the solution was collected and washed several times with water. Lactone **14** thus obtained was crystallized in methyl alcohol giving colorless crystals (6.9 g, 85%) mp: 134°C. ^1H NMR ($CDCl_3$): δ 3.66 (s, 3H, OCH_3); 3.74 (s, 3H, OCH_3); 6.63 (m, 1H, $H_{6'}$); 6.66 (s, 1H, H_3); 6.93 (m, 2H, $H_{3'}$, $H_{4'}$); 7.83 (d, 1H, H_6, $J_{6,7} = 5$ Hz); 8.66 (d, 1H, H_7). Anal. $C_{15}H_{12}ClNO_4$ (C, H, N).

2-Chloro-3-(2,5-dimethoxy benzoyl)-4-pyridine carboxylic acid ***15***

Oxidation of **14** (6.1 g, 20 mmol) by means of *N*-bromosuccinimide as described for **10** afforded yellow needles (4 g, 62%) from methyl alcohol, mp: 211°C. ^1H NMR ($[^2H_6]Me_2SO$): δ 3.42 (s, 3H, OCH_3);

3.83 (s, 3H, OCH_3); 7.10 (d, 1H, $H_{3'}$); 7.28 (m, 1H, $H_{4'}$); 7.48 (q, 1H, $H_{6'}$); 7.94 (d, 1H, H5, $J_{5,6}$ = 5 Hz); 8.65 (d, 1H, H_6). Anal. $C_{15}H_{12}ClNO_5$ (C, H, N).

1-Chloro-6,9-dihydroxy benzo[g]isoquinoline-5,10 dione **16**
Cyclization of ketoacid **15** was conducted as for **11a** by means of methane sulfonic acid to give a 57% yield of red needles (toluene), mp: 243°C. ^1H NMR ($CDCl_3$): δ 7.34 (d, 1H, H_7 or H_8, $J_{7,8}$ = 9.5 Hz); 7.46 (d, 1H, H_7 or H_8); 8.21 (d, 1H, H_4 or H_3, $J_{4,3}$ = 5 Hz); 8.88 (d, 1H, H_3 or H_4); 12.57 (s, 1H, OH); 12.40 (s, 1H, OH). Anal. $C_{13}H_6ClNO_4$ (C, H, N).

1-[[3-(Diethylamino)propyl]amino]-6,9-dihydroxy benzo[g]isoquinoline-5,10-dione **22a**
Chloro derivative **16** was dissolved in toluene and treated with 1.2 eq of 3-(diethylamino)propylamine. The mixture was refluxed under argon and the disappearance of the quinone was monitored by thin-layer chromatography (SiO_2 ethyl acetate—ethyl alcohol, 1:1). The reaction was complete after 1 h and was allowed to cool at room temperature. The solvent was removed by rotary evaporation. Hot hexane was added to the residue and the solution filtered. By cooling **22a** crystallizes as purple needles (70%) mp: 88°C. ^1H NMR ($CDCl_3$): δ 1.04 (t, 6H, ethyl CH_3); 1.86 (q, 2H, β CH_2); 2.58 (m, 6H, ethyl CH_2 and γ CH_2); 3.68 (q, 2H, α CH_2); 7.21 (d, 1H, H_7 or H_8); 7.32 (d, 1H, H_8 or H_7); 7.30 (d, 1H, H_4, $J_{4,3}$ = 5 Hz); 8.61 (d, 1H, H_3); 9.5 (1H, NH). Anal. $C_{20}H_{23}N_3O_4$ (C, H, N).

1-[[2-(Dimethylamino)ethyl]amino]-6,9-dihydroxy benzo[g]isoquinoline-5,10-dione **22b**
16 was refluxed with 1.2 eq of 2-(dimethylamino)ethyl amine in toluene solution for 3 h then the mixture stood overnight at 50°C. **22b** was extracted with 1 N hydrochloric acid and the aqueous layer was washed twice with chloroform and then adjusted to pH 7 with dilute NH_4OH. The resulting solution was extracted with $CHCl_3$ and the organic phase dried over Na_2SO_4 and evaporated under reduced pressure. The residue crystallized from cyclohexane as deep purple needles (70%) mp: 185°C. ^1H NMR ($CDCl_3$): δ 2.35 (s, 6H, CH_3); 2.64 (t, 2H, β CH_2); 3.78 (q, 2H, α CH_2); 7.22 (d, 1H, H_7 or H_8); 7.34 (d, 1H, H_8 or H_7); 7.34 (d, 1H, H_4, $J_{4,3}$ = 4.8 Hz); 8.62 (d, 1H, H_3); 9.49 (1H, NH); 12.88 (2H, OH6 and OH9). Anal. $C_{17}H_{17}N_3O_4$ (C, H, N).

1-[[2-(2-Hydroxyethyl)amino ethyl]amino]-6,9-dihydroxy benzo[g]isoquinoline 5,10-dione **22c**
The reaction of **16** with 2-(2-aminoethyl)amino ethanol was not reproducible. Several experiments were carried out under various conditions (solvent, reaction time, temperature). However, the yield of azaanthraquinone **22c** never exceeded 10%. In a typical experiment, a toluene solution of **16** was treated with 1 eq of amine at 50°C under argon for 12 h. The resulting suspension was filtered out and the filtrate extracted with 1 N HCl. The aqueous layer was neutralized with K_2CO_3 and extracted with $CHCl_3$. The organic solution was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was taken up with minimum volume of hot toluene, filtered and **22c** was precipitated with hexane as a purple solid (very hygroscopic) mp: 119°C. ^1H NMR ($CDCl_3$): δ 2.96 (m, 4H, β and δ CH_2); 3.7 (m, 4H, α and γ CH_2); 7.26 (degenerated AB, 2H, H_7 and H_8); 7.36 (d, 1H, H_4, $J_{4,3}$ = 5 Hz); 8.63 (d, 1H, H_3); 9.6 (bs, 1H, exchangeable). Anal. $C_{17}H_{17}N_3O_5$ (C, H, N).

In most cases, especially with butyl alcohol as the solvent, the dimeric compound mentioned in the text was obtained as the major reaction product (up to 45%): purple red powder mp > 280°C; m/z = 583 (DCI with NH_3); Anal. $C_{30}H_{22}N_4O_9$; H_2O (C, H, N).

2-[2-Chloro-3(α-hydroxy-α-(1,4,8-trimethoxy-2-naphthyl)methyl)-4-pyridyl]-4,4-dimethyl 4,5-dihydrooxazole **18**
Lithiation of 2-(2 chloro-4-pyridyl)-4,4-dimethyl-4,5-dihydrooxazole **12** (5 g, 23 mmol) in dry tetrahydrofuran (THF) (100 ml) at —70°C under nitrogen by means of methyl lithium (32.6 ml of a 0.8 M solution in ether, 1.1 mmol eq) and subsequent condensation with 1,4,8-trimethoxy-2-naphthaldehyde [14] (5,6 g, 23 mmol) in THF (30 ml) afforded, after usual treatment, **18**, 5.5 g, 52% of light yellow crystals from toluene. NMR spectrometry shown, as for **13**, a partial opening of the oxazoline ring.

1,3-Dihydro-3-(1,4,8-trimethoxy-2-naphthyl)-4-chloro (3H) pyrido[3,4-c]furan-1-one **19**
Because hydrochloric acid hydrolysis of **18** led to partial demethylation, acetic acid was used for preparative purposes. Thus, **18** (5.5 g, 12 mmol) was dissolved in acetic acid (50 ml) and the solution was stirred at room temperature for 24 h and then diluted with water. The precipitate was collected by filtration to give 3.2 g (69.5%) of a white crystalline powder from ethyl alcohol mp: 195°C. ^1H NMR ($CDCl_3$): δ 3.78 (s, 3H, OMe); 3.89 (s, 3H, OMe); 4.04 (s, 3H, OMe); 6.05 (s, 1H, H_3); 6.99 (q, 1H, $H_{7'}$); 7.13 (s, 1H, $H_{3'}$); 7.47 (t, 1H, $H_{6'}$); 7.88 (m, 1H, $H_{5'}$); 7.85 (d, 1H, H_6, $J_{6,7}$ = 5 Hz); 8.71 (d, 1H, H_7). Anal. $C_{20}H_{16}ClNO_5$ (C, H, N).

2-Chloro-3-(1,4,8-trimethoxy-2-naphthoyl)-4-pyridine carboxylic acid **20**
Oxidation of **19** with NBS and subsequent hydrolysis as for **9** and **14** afforded 1.3 g (42%) of yellow needles from methanol mp: 232°C. ^1H NMR ($[^2H_6]Me_2SO$): δ 3.19 (s, 1H, OMe); 3.36 (br, s, 1H, exchangeable, OH); 3.94 (s, 3H, OMe); 4.06 (s, 3H, OMe); 7.17 (q, 1H, $H_{7'}$); 7.41 (s, 1H, $H_{3'}$); 7.65 (t, 1H, $H_{6'}$); 7.85 (m, 1H, $H_{5'}$); 7.96 (d, 1H, H_5, $J_{5,6}$ = 5 Hz); 8.68 (d, 1H, H_6). Anal. $C_{20}H_{16}ClNO_6$ (C, H, N).

1-Chloro-6,10,11-trihydroxynaphtho[2,3-g]isoquinoline 5,12 dione **21**
Cyclization of **20** in methane sulfonic acid following the standard procedure and subsequent chromatography over SiO_2, eluting with a 1:1 mixture of xylene and dichloromethane, afforded a 20% yield of bright red plates from xylene, mp: 295°C. ^1H NMR ($CDCl_3$): δ 7.32 (q, 1H, H_8); 7.76 (t, 1H, H_9); 7.98 (m, 1H, H_7); 8.28 (d, 1H, H_4 or H_3, $J_{4,3}$ = 5 Hz); 8.65 (d, 1H, H_3 or H_4); 12.19 (s, 1H, OH); 14.26 (s, 1H, OH_{11} or OH_6); 14.72 (s, 1H, OH_6 or OH_{11}). Anal. $C_{17}H_8ClNO_5$ (C, H, N).

1-[[3-(Diethylamino)propyl]amino]-6,10,11-trihydroxy naphtho[2,3-g]isoquinoline 5,12 dione **23**
A toluene solution of 1 eq of **21** with 1.2 eq of 3-(diethylamino)propylamine was heated at 100°C for 8 h under argon. The work up as for **22a** afforded **23** (85%) as purple needles from cyclohexane, mp: 182°C. ^1H NMR ($CDCl_3$): δ 1.13 (t, 6H ethyl CH_3); 1.97 (m, 2H β CH_2); 2.70 (q, 6H, γ CH_2 and ethyl CH_2); 3.7 (t, 2H α CH_2); 7.23 (t, 1H, H_7); 7.64 (t, 1H, H_8); 7.9 (q, 1H, H_9); 7.34 (d, 1H, H_4, $J_{4,3}$ = 4.8 Hz); 8.33 (d, 1H, H_3). Anal. $C_{24}H_{25}N_3O_5$ (C, H, N).

Biological assays

Cell culture and in vitro *cytostatic activity*
Friend tumor cells (clone 745) were grown in suspension in MacCoy medium supplemented with 10% fetal calf serum. L1210 cells were grown in suspension in RPMI 1640 medium supplemented with 10% calf serum.

On day 0, 10^5 cells were plated in a volume of 1 ml in sterile Limbro disposable plates. On day 1, cultures were in the exponential phase of growth and increasing dilutions of the test drugs dissolved in dilute acetic acid and adjusted to pH 7 with sodium hydrogenocarbonate were added in duplicate cultures in a volume of 10 μl. 24 h later, cell counts were made using a Malassez hemocytometer and cell viability was estimated by the trypan blue dye exclusion test. ID_{50} were determined from the regression line of inhibition value obtained at various concentrations.

In vivo *studies*
10^6 P388 cells were inoculated intraperitoneally into female CDF_1 mice in a volume of 0.1 ml on day 0. Test compounds in 0.01 M HCl were injected by the same route on days 1—5, at doses ranging from 10 to 40 mg/kg. Control experiments were carried out with 1.5 mg/kg of adriamycin and 20 mg/kg 5-fluorouracil. Untreated controls received only 0.01 M HCl.

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