Synthesis of 1-Functionalized-6-hydroxy-4-methyl and 6,11-Dihydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinones

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Using 2-methoxy- and 2,5-dimethoxyacetophenones **8a** and **8b** as starting materials, 1-chloro-4-methylisoquinoline-5,8-quinone (**6**) and its 6-bromo derivative **7** were obtained *via* multistep sequences. Whereas Diels-Alder condensation of the former compound with homophthalic anhydride (**22**) led to a mixture of the two possible isomers: 1-chloro-11-hydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinone (**23**) and 1-chloro-6-hydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinone (**24**), this last tetracyclic chloroquinone was specifically obtained from 6-bromo-1-chloro-4-methylisoquinoline-5,8-quinone (**7**) and homophthalic anhydride. The 6,11-dihydroxy derivative was then prepared by ammonium nitrate oxidation or photochemically by cycloaddition of benzocyclobutenedione (**28**) and 1-chloro-4-methylisoquinoline-5,8-quinone (**6**). Chloro compounds were easily substituted by diamines to provide corresponding 1-amino substituted hydroxy tetracyclic quinones.

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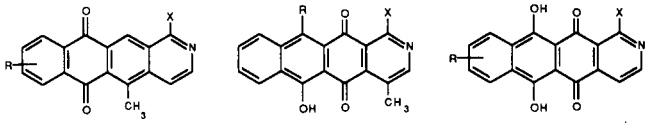
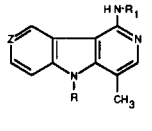
In a recent paper, we described the synthesis of 1-functionalized-5-methylnaphtho[2,3-*g*]isoquinoline-6,11-quinones **1** which had been obtained through two independant pathways [1]. Our continuous interest in the field of heterocyclic quinones related to antitumor anthraquinones bearing dibasic side chains led us to design 1-functionalized-6-hydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinones **2** as new target molecules.

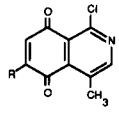


1 2 3

In spite of the fact that these products are closely related to azanaphthacene quinones **3** which are devoid of cytostatic activity [2], our results in the pyrido[3',4':4,5]-pyrrolo[3,2-*c*]pyridine series **4** and γ-carbolines series **5**, where the presence of the 4-methyl group clearly confer cytostatic and antitumor properties [3,4] seemed to us important enough to justify a specific search devoted to this class of polycyclic quinone derivatives.



4: Z = N **5**: Z = C-OH
$R_1 = (CH_2)_3 - N(CH_3)_2$
R = H or CH_3



6: R = H
7: R = Br

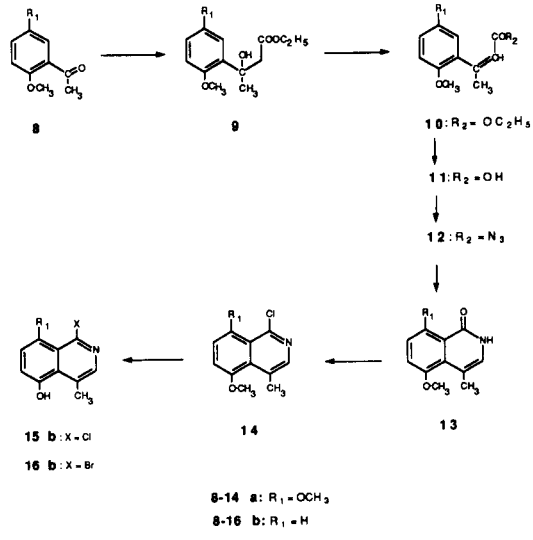
In this report, we describe the synthesis which allowed us to obtain 1-amino substituted-4-methyl-tetracyclic derivatives **2**.

Chemistry.

As we reported in a preceding paper, Diels-Alder reaction of isoquinoline-5,8-quinones with 1,4-diacetoxy-1,3-butadiene led to benzo[*g*]isoquinoline-5,10-quinones [5]. To obtain 1-functionalized-6-hydroxy (and 11-hydroxy)-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinones, it seemed thus possible to react homophthalic anhydride with 1-chloro-4-methylisoquinoline-5,8-quinone itself (**6**) or with its 6-bromo derivative **7** for a more regiospecific condensation. We then undertook the synthesis of these two isoquinolinequinones.

Starting from 2,5-dimethoxyacetophenone (**8a**), Reformatzki condensation with ethyl bromoacetate and subsequent dehydration and saponification successively led to

Scheme I



8 **9** **10**: $R_2 = OC_2H_5$
11: $R_2 = OH$
12: $R_2 = N_3$
13 **14**
15 b: X = Cl
16 b: X = Br
8-14 a: $R_1 = OCH_3$
8-16 b: $R_1 = H$

ethyl 3-hydroxy-3-(2,5-dimethoxyphenyl)butyrate (**9a**), ethyl 3-(2,5-dimethoxyphenyl)crotonate (**10a**) and the corresponding crotonic acid **11a**. After transformation into the azide **12a** by the usual mixed anhydride method, the Eloy and Deryckere [6] thermal rearrangement-heterocyclisation then gave isoquinolone **13a**, easily transformed into 1-chloro-5,8-dimethoxy-4-methylisoquinoline (**14a**) (Scheme I).

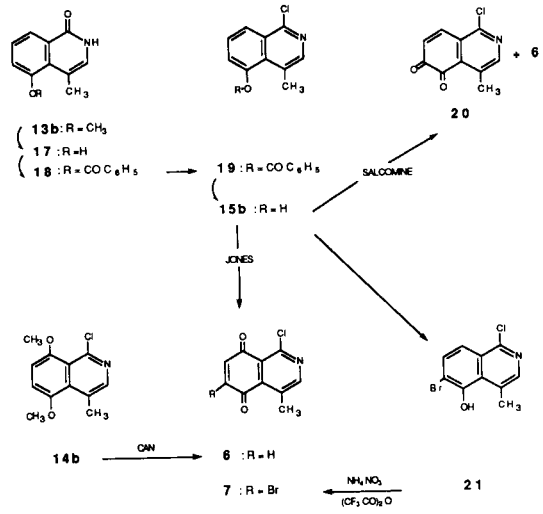
Oxidative demethylation of this last compound with cerium ammonium nitrate (CAN) [7] provided the desired 1-chloro-4-methylisoquinoline-5,8-quinone (**6**) in 90% yield (Scheme II). However, especially due to the low yield in **12a**→**13a** transformation (10%), the overall yield of this six step synthesis did not exceed 5%. For this reason the same reactions were performed starting from 2-methoxyacetophenone (**8b**). In that case, **12b**→**13b** yield was then increased to 41% and 1-chloro-4-methyl-5-methoxyisoquinolines **14b** [8] was obtained with a 23% overall yield.

We first tried to prepare compound **15b** by hydrobromic acid demethylation of **14b**. This reaction was accompanied by a partial chlorine to bromide exchange and a mixture of **15b** and 1-bromo-5-hydroxy-4-methylisoquinoline (**16b**) was obtained which was difficult separate (Scheme I). Since other demethylation methods, such as boiling pyridine hydrochloride and boron trichloride in dichloromethane failed, pure 1-chloro-5-hydroxy-4-methylisoquinoline (**15b**) was synthesized from isoquinolone **13b** *via*; i) boiling pyridine hydrochloride demethylation to 5-hydroxy-4-methyl-2*H*-isoquinolin-1-one (**17**); ii) benzoylation to **18** for protecting of hydroxy group; iii) chlorination by phosphorus oxychloride to give **19**; iiii) saponification to free phenol **15b**, which was thus obtained with a 16% overall yield (Scheme II).

Disappointing results were obtained when oxidation of 1-chloro-5-hydroxy-4-methylisoquinoline (**15b**) was studied under various conditions. Thus: i) Salcomine catalyzed oxidation [9] led to a total 75% yield of a *circa* 1/1 mixture of **6** and 1-chloro-4-methylisoquinoline-5,6-quinone (**20**), as shown by nmr spectroscopy. Unfortunately, attempts to resolve this mixture by column or preparative thin layer chromatography on silica gel totally failed; ii) sodium bichromate in sulfuric acid medium, as well as Jones reagent [10] in acetone as solvent, afforded a complex mixture from which no pure compound has been characterized; iii) Jones reagent gave the expected quinone **6** with a 10% yield in diethylether and with a 14% yield in methylene chloride (Scheme II).

As a conclusion of this study, it must be pointed out that the second pathway used for 1-chloro-4-methylisoquinoline-5,8-quinone (**6**) preparation was not better than was the preceding one. Reaction of 1-chloro-4-methylisoquinoline-5,8-quinone (**6**) with homophthalic anhydride [22] led to a mixture of the two possible isomers: 1-chloro-11-hydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinone (**23**) and 1-chloro-6-hydroxy-4-methylnaphtho[2,3-*g*]isoquinoline (**24**), with a total yield of 77%. This mixture was not resolved in the pure compounds by column chromatography and ^{1}H-nmr spectrum did not allow us to identify each product.

Scheme II

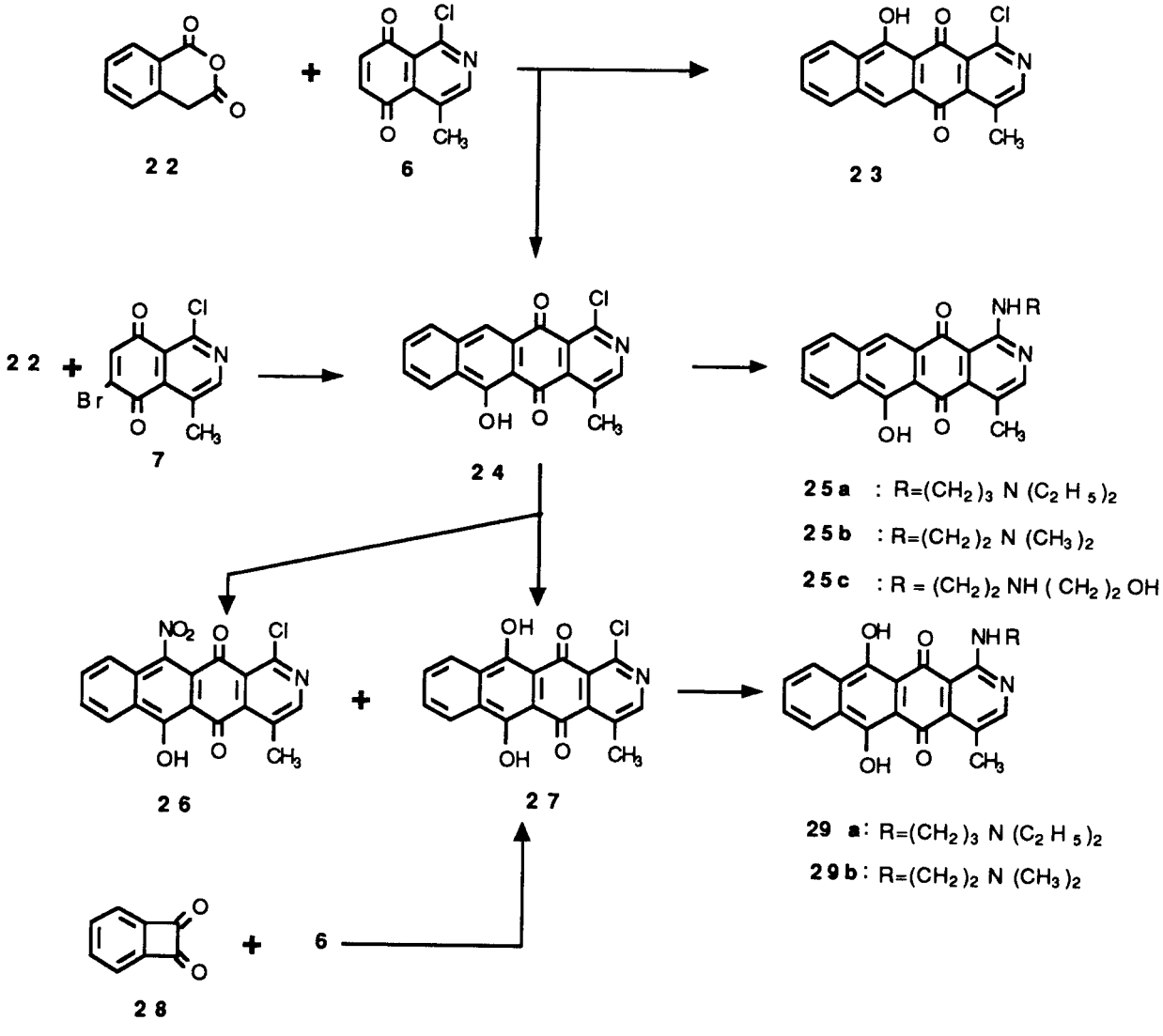


As demonstrated by Tamura [11] regiospecific Diels-Alder condensation can be obtained using a bromoquinone derivative. We then prepared 6-bromo-1-chloro-6-hydroxy-4-methylisoquinoline (**21**) by reaction of 2,4,4,6-tetrabromocyclohexan-2,5-dien-1-one [12] with hydroxyisoquinoline **15b**, in 70% yield. Oxidation of this compound was then studied under various conditions: i) potassium nitrosodisulfonate (Fremy's salt) did not transform compound **21**; ii) Jones reagent gave traces of the expected bromoquinone **7**; iii) oxidation with ammonium nitrate in trifluoroacetic anhydride [13] gave 6-bromo-1-chloro-4-methylisoquinoline-5,8-quinone (**7**) with 15% yield (Scheme II).

As expected, condensation of homophthalic anhydride with 6-bromo-1-chloro-4-methylisoquinoline-5,8-quinone (**7**), gave a single isomer. The ^{1}H-nmr spectrum of the compound obtained under these conditions unambiguously corresponded to structure **24**, in agreement with reported data related to the addition of unsymmetrical bromoquinones with homophthalic anhydrides [11]. After **24** identification and coming back to **23** + **24** mixture precedingly obtained, the corresponding spectrum was then analysed on the basis of 6- and 11-proton signal integrations. This showed that isoquinolinequinone **6** and homophthalic anhydride react giving **23** + **24** in a ratio of 75/25 (Scheme III). Although unsatisfactory, there is thus a marked regioselectivity for isomer **23** formation.

For completion of our work, **24** was reacted with ammonium nitrate-trifluoroacetic anhydride mixture [13], giving nitro derivative **26** and the expected 1-chloro-6,11-

Scheme III



dihydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinone (**27**) (10%). Under the same conditions, however, this last compound was not islated when we tried to use the unresolved **23** + **24** mixture as starting material which afforded an unexploitable complex mixture.

In order to obtain tetracyclic chloroquinone **27**, we tried a photochemically induced cycloaddition of the bisketene generated from benzocyclobutene dione (**28**) [14,15], to chloroisoquinolinequinone **6**, as recently described for a synthesis of Daunomycinone derivatives [16]. This reaction took place, as expected, but unfortunately, only a 7% yield of **27** was obtained, despite various experiments with different ratio of the starting materials. Finally, compound **24** was reacted with 3-diethylaminopropylamine, 2-dimethylaminoethylamine and 2-(2-hydroxyethyl)aminoethylamine under usual conditions. The corresponding 1-aminosubstituted-6-hydroxy-4-methylnaphtho[2,3-*g*]-isoquinoline-5,12-quinones **25a-c** were thus obtained, as well as compounds **29a-b** from **27** and the appropriate amines (Scheme III).

Biological Evaluation.

Growth inhibition of various cultured tumor-cell lines (Friend 745, L1210, P388) were determined following the standard NCI protocols. As shown in Table 1, compounds **25a** was found slightly active against P388 (IC_{50} = $10^{-6}M$) but inactive in other two cell lines: compounds **25b** and **25c** had IC_{50} between 2 and 5 x 10^{-6} *M* on all tested cell lines but were toxic at higher doses (as well as **25a** was). Dihydroxy derivatives **29a** and **29b** were tested in the same systems and results show that they are either toxic or inactive *in vitro* (Table 1). Despite these disappointing results, the five new derivatives were tested *in vivo* in the P388 leukemia system, in comparison with a reference aza-

Table 1
Results of Biological Studies

Compound	*in vitro* - IC 50 after 24 hours (μM) L1210	P388	745	in vivo [a] P388 - 6 ♀ + 6 ♂ for every test group T/C (mg/kg) J_1 (60 days survivors)
Reference compound BD40	0.026	0.017	0.015	130 (20) (2)
25a	inactive [b]	1	3	102 (30) (0)
25b	5	2	4	106 (30) (0)
25c	inactive [b]	2.5 [c]	2	102 (30) (0)
29a	inactive [b]	2	toxic [d]	113 (30) (0)
29b	6.5	toxic [d]	toxic [d]	113 (30) (0)

[a] 10^6 P388 cells were inoculated intrapertaneally (i.p.) at day 0. Test compounds were admnistered i.p. at D1 in 0.01*M*-HCl. [b] Inactive at 1 μ*M*. Not tested at higher concentration. [c] Extrapolated value from regression curve, but toxic at 5 μ*M*. [d] Inactive at 1 μ*M* but toxic at 5 μ*M*.

ellipticine compound (BD40) [17]. As shown in Table 1, these experiments clearly confirm that compounds **25a-c** and **29a-b** do not display antitumor properties.

Conclusion.

In the series of naphtho[2,3-*g*]isoquinoline-5,12-quinones, the presence of a 4-methyl group does not enhance cytotoxicities toward tumor cultured cells, as it does in the tricyclic series **4** and **5**. Taking into account our preceding reports and the present one, various 1-amino-substituted-naphtho[2,3-*g*]isoquinolinequinones have thus been synthesized and studied for their possible antitumor properites. By comparison with tetracyclic derivatives related to antitumor ellipticine and analogues, and tricyclic series **4** and **5** as well, the lack of important cytotoxicities and antitumor properties for all the newly studied heterocyclic quinone series must be pointed out.

EXPERIMENTAL

All melting points were determined with a Kofler apparatus and are uncorrected. The nmr spectra were recorded with a Varian XL 100, at 100 MHz. TMS was used as an internal standard, and chemical shifts were reported on the δ scale with peak multiplicities. Elemental analysis were performed by Service Central de Microanalyses du CNRS, 91190 Gif sur Yvette, France. Mass spectra were measured on a NERMAG 70 eV with direct introduction.

3-(2,5-Dimethoxyphenyl)crotonic Acid (**11a**).

To a mixture of 2,5-dimethoxyacetophenone **8a** (25.5 g, 0.14 mole), activated zinc (12 g, 0.18 mole) in dry benzene (120 ml) maintained at reflux, ethyl 2-bromoacetate (16 g, 0.14 mole) was added dropwise with vigourous stirring, at such a rate that reflux was maintained without further heating. Reflux was pursued 1 hour after addition was completed. After cooling, the mixture was poured into diluted (0.5 *N*) hydrochloric acid and extracted with toluene (3 x 100 ml). The combined extracts were washed with water (3 x 100 ml), dried over magnesium sulfate and evaporated, giving an oily residue (29.8 g, 79%) of crude hydroxyester **9a** which was directly used for the next step.

Dehydration was performed in acetic acid (100 ml) containing 6*N*-hydrochloric acid (1 ml) at reflux for 2 hours. After evaporation of acetic acid, the residue was taken up in water, extracted with methylene chloride, worked up as usual and distilled to afford 25 g (90%) of an oil (bp = 132°/0.3 mm) corresponding to the ester **10a** (mixture of *cis* and *trans* isomers). This mixture (25 g, 0.1 mole) was treated with potassium hydroxide (8.5 g, 0.15 mole) dissolved in 95% ethanol (150 ml) at reflux for 2 hours with stirring. Ethanol was removed and the residue was dissolved in water, then acidified and extracted with methylene chloride (3 x 150 ml). The combined extracts were dried over magnesium sulfate and evaporated giving a residue which was taken up in boiling hexane to afford 19.5 g (88%) of colorless crystals, mp 115° corresponding to a 8/2 mixture of *cis* and *trans*-isomers of crotonic acid **11a**; ^{1}H nmr (deuteriochloroform): δ 2.18 (d, CH_3-*cis*, J_{CH_3}-C*H* = 1.5 Hz) 2.51 (d, CH_3-*trans*, J_{CH_3}-C*H* = 1.5 Hz), 3.76 (4s, 2,5-(OCH_3)-*cis* + 2,5 (OCH_3)-*trans*), 5.35 (d, C*H* cis), 5.95 (d, C*H* trans), 6.74 (m, H-Ar), 11.5 (br s, COO*H*).

Anal. Calcd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 65.13; H, 6.41.

5,8-Dimethoxy-4-methyl-2*H*-isoquinolin-1-one (**13a**).

Ethyl chloroformate (8 ml, 80 mmoles) dissolved in acetone (120 ml) was added dropwise to a stirred solution of the preceding acid **11a** (15.6 g, 70 mmoles) in acetone (120 ml) and triethylamine (11.5 ml, 80 mmoles) cooled and maintained at -10°. One hour after addition was complete, sodium azide (7.2 g, 110 mmoles) dissolved in the minimum amount of water was added dropwise at the same temperature. Stirring was pursued for 2 hours allowing the mixture to reach room temperature. Acetone was removed at 25° under reduced pressure and the oily residue was poured into water, extracted with methylene chloride (3 x 100 ml) and washed with water (3 x 50 ml). The dried extract (magnesium sulfate) was evaporated at room temperature under reduced pressure, giving an oily residue (16 g) of crude azide **12a** which was kept cold and used for the next step without further purification. Thus, a mixture of diphenyl ether (90 ml) and tributylamine (15.5 ml, 65 mmoles) was placed in a three necked flask fitted with a mechanical stirrer and heated at 240-245°. Then, the solution of the preceding crude azide **12a** (16 g, 65 mmoles) in diphenyl ether (90 ml) was progressively added. Temperature (240-250°) and stirring were maintained for 4 hours and a part (140 ml) of diphenyl ether was evaporated under reduced pressure. Hexane (150 ml) was added to the cooled residue, giving two layers, which were separated by decantation. The oil was taken up in toluene and stirred until a yellow solid appeared. After filtration, it was recrystallized from toluene to provide 1.5 g (10%) of yellow crystals corresponding to the expected isoquinolone **13a**, mp 225°; ^{1}H nmr (deuteriochloroform): δ 2.45 (d, 3H, 4-CH_3, $J_{CH_3}H_3$ = 0.9 Hz), 3.97 + 4.11 (2s, 2 x 3H, 5,8-(OCH_3)$_2$), 7.03 (d, 1H, 3-H), 7.01 (d, 1H, 7-H, J_{7-6} = 8.8 Hz), 7.12 (d, 1H, 6-H), 11.58 (br s, 1H, NH).

Anal. Calcd. for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.97; N, 6.38. Found:

C, 65.57; H, 5.81; N, 6.42.

1-Chloro-5,8-dimethoxy-4-methylisoquinoline (**14a**).

Isoquinolone **13a** (1.1 g, 5 mmoles) was heated in phosphorus oxychloride (50 ml) at reflux for 2 hours and the excess of phosphorus oxychloride was evaporated under reduced pressure. The residue was treated with ice water, basified with potassium carbonate and the resulting mixture was extracted with methylene chloride (3 x 100 ml). The combined extracts were dried over magnesium sulfate and the residue obtained by evaporation was chromatographed on a silica gel column, eluting with methylene chloride. The solid obtained was recrystallized from cyclohexane to give chloroisoquinoline **14a** (72%) as yellow needles, mp 127°; ^{1}H nmr (deuteriochloroform): δ 2.73 (s, 3H, 4-CH_3), 3.87 + 3.93 (2s, 2 x 3H, 5,8-$(OCH_3)_2$), 6.93 (m, 2H, 6-H + 7-H), 7.96 (s, 1H, 3 - H).

Anal. Calcd. for $C_{12}H_{12}ClNO_2$: C, 60.63; H, 5.08; N, 5.89. Found: C, 60.65; H, 5.18; N, 5.85.

3-(2-Methoxyphenyl)crotonic Acid (**11b**).

This compound was synthesized as described above for obtaining crotonic acid **11a** but starting from 2-methoxy-acetophenone. The overall yield was 64% for the 7/3 mixture of *trans* + *cis* isomers, mp 95°.

Anal. Calcd. for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29; O, 24.97. Found: C, 68.70; H, 6.38; O, 24.83.

5-Methoxy-4-methyl-2*H*-isoquinolin-1-one (**13b**).

Transformation of acid **11b** (80 g, 0.41 mole) to the crude azide **12b** (80 g, 89%) and thermal cyclisation were performed according to procedures described above for obtaining compound **13a**. Isoquinolone **13b** was obtained as a solid which was recrystallized from ethanol to afford 26 g (46%) of colorless needles, mp 216°; ^{1}H nmr (deuteriochloroform): δ 2.48 (d, 3H, 4-CH_3, J_{CH_3}-3-H = 0.9 Hz) 3.91 (s, 3H, 5-OCH_3) 6.81 (d, 1H, 3-H), 7.13 (m, 1H, 6-H, J_{6-7} = 8 Hz J_{6-8} = 1.2 Hz), 7.44 (t, 1H, 7-H, J_{7-8} = 7.9 Hz), 8.10 (dd, 1H, 8-H) 9.5 (br s, 1H, NH).

Anal. Calcd. for $C_{11}H_{11}NO_2$: C, 69.82; H, 5.86; N, 7.40. Found: C, 69.86; H, 5.86; N, 7.43.

1-Chloro-5-methoxy-4-methylisoquinoline (**14b**).

Using the technique described above for obtaining chloroisoquinoline **14a**, chlorination of isoquinolone **13b** afforded a solid which was recrystallized from ethanol giving a 91% yield of compound **14b** as colorless needles, mp 123°; ^{1}H nmr (deuteriochloroform): δ 2.77 (d, 3H, 4-CH_3, J_{CH_3}-3-H = 0.5 Hz), 3.97 (s, 3H, 5-OCH_3), 7.06 (dd, 1H, 6-H, J_{6-7} = 7.9 Hz, J_{6-8} = 1 Hz), 7.56 (t, 1H, 7-H, J_{7-8} = 8.3 Hz) 7.95 (dd, 1H, 8-H), 7.99 (d, 1H, 3-H).

Anal. Calcd. for $C_{11}H_{10}ClNO$: C, 63.62; H, 4.85; N, 6.74. Found: C, 63.80; H, 4.88; N, 6.88.

5-Hydroxy-4-methyl-2*H*-isoquinolin-1-one (**17**).

A mixture of pyridinium chloride (100 g, 0.85 mole) and isoquinolone **13b** (10 g, 53 mmoles) was refluxed for 1 hour and poured into water after cooling. The solution was basified with an excess of aqueous 1*N* sodium hydroxide, hot filtered and acidified with acetic acid. The solid was filtered off and recrystallized from acetic acid to afford 8.5 g (92%) of colorless needles, mp >300°; ^{1}H nmr (DMSO-d_6): δ 2.44 (d, 3H, 4-CH_3, J_{CH_3}-3-H = 0.9 Hz), 6.76 (br s, 1H, 3-H), 7.10 (dd, 1H, 6-H, J_{6-7} = 7.2 Hz, J_{6-8} = 2 Hz), 7.27 (t, 1H, 7-H, J_{7-8} = 8Hz), 7.72 (dd, 1H, 8-H), 9.87 (s, 1H, OH), 10.95 (br s, 1H, NH).

Anal. Calcd. for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.49; H, 5.07; N, 8.08.

5-Benzoyloxy-4-methyl-2*H*-isoquinolin-1-one (**18**).

A mixture of the preceding hydroxyisoquinolone **17** (8 g, 45 mmoles), dry pyridine (250 ml) and benzoic anhydride (30.5 g, 135 mmoles, 3 equivalents) was heated at reflux for 2 hours and pyridine was evaporated under reduced pressure. The residue was taken up in water (200 ml) and acetone (2 ml) then a little excess of sodium hydrogen carbonate was added and the resulting mixture was stirred for 1 hour. The solid was collected, washed with water and hot ethanol and air dried, giving 11.5 g (90%) of colorless microcrystals, mp 278°; ^{1}H nmr (deuteriochloroform): δ 2.32 (s, 3H, 4-CH_3), 6.84 (br s, 1H, 3-H), 7.56 (m, 5H, 7-H + 6-H + 3H -Ar), 8.26 (dd, 2H, 2-H + 6-H-Ar) 8.46 (dd, 1H, 8-H, J_{8-7} = 7.8 Hz, J_{8-6} = 2Hz), 9.80 (br s, 1H, NH).

Anal. Calcd. for $C_{17}H_{13}NO_3$: C, 73.11; H, 4.69; N, 5.02. Found: C, 72.89; H, 4.72; N, 4.86.

5-Benzoyloxy-1-chloro-4-methylisoquinoline (**19**).

Compound **18** (10 g, 35 mmoles) was heated in phosphorus oxychloride (200 ml) at reflux for 2.5 hours and an excess of phosphorus oxychloride was removed under reduced pressure. The residue was poured into ice-water and the aqueous solution was neutralized with sodium hydrogen carbonate. The resulting solid was filtered off, washed with water and recrystallized from ethanol to afford 8.5 g (80%) of colorless microcrystals, mp 158°; ^{1}H nmr (deuteriochloroform): δ 2.6 (d, 3H, 4-CH_3, J_{CH_3}-3-H = 1Hz), 7.73 (m, 5H, 7-H + 6-H + 3H-Ar), 8.05 (q, 1H, 3-H), 8.26 (m, 2H, 2-H + 6-H-Ar) 8.38 (dd, 1H, 8-H, J_{8-7} = 8.3 Hz, J_{8-6} = 1.4 Hz).

Anal. Calcd. for $C_{17}H_{12}ClNO_2$: C, 68.57; H, 4.06; N, 4.70. Found: C, 68.54; H, 4.04; N, 4.62.

1-Chloro-5-hydroxy-4-methylisoquinoline (**15b**).

a) Demethylation of methoxyisoquinoline **14b** in boiling hydrobromic acid (48%, d = 1.47, 6 hours, 4 hours, 2 hours) afforded a mixture of 1-bromo-5-hydroxy-4-methylisoquinoline **16b** and 1-chloro-derivative **15b** which has not been resolved into the pure compounds.

b) Benzoyloxyisoquinoline **19** (8.5 g, 28 mmoles) was stirred at room temperature overnight in 225 ml of methanol saturated with ammonia and evaporated to dryness. The residue was triturated in water and the solid filtered and air dried. It was recrystallized from toluene affording 5.3 g (96%) of colorless needles, mp 234°; ^{1}H nmr (deuterioacetone): δ 2.83 (d, 3H, 4-CH_3, J_{CH_3}-3-H = 1.1 Hz) 7.25 (dd, 1H, 6-H, J_{6-7} = 7.6 Hz, J_{6-8} = 1,3 Hz), 7.55 (t, 1H, 7-H, J_{7-8} = 8.4 Hz), 7.83 (dd, 1H, 8-H), 7.96 (d, 1H, 3-H).

Anal. Calcd. for $C_{10}H_8ClNO$: C, 62.03; H, 4.16; N, 7.23. Found: C, 62.04; H, 4.15; N, 6.94.

1-Chloro-4-methylisoquinoline-5,8-quinone (**6**).

Method A.

Hydroxyisoquinoline **15b** (1 g, 3.5 mmoles) was dissolved in dichloromethane (500 ml) and 5 ml of Jones reagent (prepared from sodium bichromate-dihydrate (165 g, 0.55 mole) 96% sulfuric acid (105 ml, 1 mole) and water (235 ml)) was added dropwise at room temperature with stirring. The mixture was kept under stirring at room temperature, for 18 hours. The organic phase was separated and evaporated. The residue was chromatographed on a silica gel column, eluting with dichloromethane. Evaporation of the pure compound containing fractions afforded

a solid which was recrystallized from cyclohexane to give 150 mg (14%) of pale yellow needles, mp 150°; ^{1}H nmr (deuteriochloroform): δ 2.71 (s, 3H, 4-CH_3), 6.97 (s, 2H, 6-H + 7-H), 8.62 (s, 1H, 3-H).

Anal. Calcd. for $C_{10}H_6ClNO_2$: C, 57.85; H, 2.91; N, 6.74. Found: C, 57.76; H, 2.95; N, 6.69.

Method B.

A cooled solution of cerium ammonium nitrate (CAN, 2.30 g, 4.21 mmoles) in a mixture of acetonitrile (4 ml) and water (4 ml) was added under vigourous stirring to an ice cooled solution of dimethoxy-isoquinoline **14a** (0.4 g, 1.68 mmoles) in acetonitrile (12 ml) and water (5 ml) containing suspended 2,6-pyridinedicarbocyclic acid *N*-oxide (0.77 g, 4.21 mmoles). After the addition was completed, stirring was maintained for 15 minutes and the mixture was poured into water and extracted with chloroform (3 x 100 ml). The combined extracts were washed with water (3 x 100 ml), dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed as in method A, to afford 300 mg (90%) of yellow needles, in all respects identical to the compound obtained by method A.

6-Bromo-1-chloro-5-hydroxy-4-methylisoquinoline (**21**).

Hydroxyisoquinoline **15b** (5 g, 25 mmoles) was dissolved in the mixture chloroform-methanol 9/1, v/v, (250 ml) and a solution of 2,4,4,6-tetrabromocyclohexa-2,5-dien-1-one [12] (10.6 g, 25 mmoles) in 250 ml of the same solvent was added dropwise at room temperature with stirring, over a 1 hour period. Stirring was pursued 30 minutes and the solvent was evaporated under reduced pressure. The residue was taken up in aqueous 1*N*-sodium hydroxide, filtered and acidified with acetic acid. The resulting solid was suspended and stirred in diluted ammonia, for 1 hour, filtered off and air dried. It was recrystallized from toluene to afford 5.1 g (72%) of colorless needles, mp 220°; ^{1}H nmr (deuteriochloroform): δ 2.83 (d, 3H, 4-CH_3, J_{CH_3-3-H} = 1 Hz), 6.24 (s, 1H, OH), 7.69 (d, 1H, 7-H, J_{7-8} = 9.3 Hz), 7.86 (d, 1H, 8-H), 8.06 (d, 1H, 3-H).

Anal. Calcd. for $C_{10}H_7BrClNO$: C, 44.07; H, 2.59; N, 5.14. Found: C, 44.26; H, 2.64; N, 4.96.

6-Bromo-1-chloro-4-methylisoquinoline-5,8-quinone (**7**).

To a suspension of bromochloroisoquinoline **21** (400 mg, 1.46 mmoles) and ammonium nitrate (586 mg, 7.3 mmoles) in dichloromethane (40 ml) of trifluoroacetic anhydride (4 ml, 27.7 mmoles) was added dropwise with stirring. The mixture was stirred at room temperature for 1.45 hours, poured into ice-water, neutralized with saturated aqueous sodium hydrogen carbonate and extracted with dichloromethane (3 x 50 ml). The combined extracts were washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed on neutral alumina Grad II (PROLABO) eluting with dichloromethane, to give 65 mg (15%) of yellow needles, mp 141°; ^{1}H nmr (deuteriochloroform): δ 2.73 (d, 3H, 4-CH_3, J_{CH_3-3-H} = 0.4 Hz), 7.52 (s, 1H, 6-H), 8.64 (d, 1H, 3-H).

Anal. Calcd. for $C_{10}H_5BrClNO_2$: C, 41.92; H, 1.75; N, 4.88. Found: C, 41.83; H, 2.09; N, 4.66.

1-Chloro-6-hydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinone **24**.

Homophthalic anhydride (850 mg, 5.24 mmoles) was dissolved in tetrahydrofuran (100 ml) under argon and cooled to 0°. Sodium hydride (60% in mineral oil, 210 mg, 5.25 mmoles) was added and after 5 minutes stirring, a solution of 6-bromo-1-chloro-4-methylisoquinoline-5,8-quinone **7** in dry THF (40 ml) was added. Stirring was continued for 30 minutes at 0° and the mixture was allowed to reach room temperature (1.5 hours). It was then quenched with a saturated solution of ammonium chloride (30 ml), acidified to *p*H 1 with 3*H*-hydrochloric acid and extracted with toluene. The combined extracts were washed with water, dried over sodium sulfate and evaporated under reduced pressure. The residue was recrystallized from toluene to provide 1.3 g (77%) of orange needles, mp >260°; ^{1}H nmr (deuteriochloroform): δ 2.90 (d, 3H, 4-CH_3, J_{CH_3-3-H} = 0.7 Hz), 7.75 (m, 2H, 8-H + 9-H), 8.04 (m, 1H, 10-H), 8.27 (s, 1H, 11-H), 8.55 (m, 1H, 7-H), 8.64 (d, 1H, 3-H), 14.23 (s, 1H, OH).

Anal. Calcd. for $C_{18}H_{10}ClNO_3$: C, 66.78; H, 3.11; N, 4.32. Found: C, 66.62; H, 3.40; N, 4.29.

1-[[3-(Diethylamino)propyl]amino]-6-hydroxy-4-methylnaphtho-[2,3-*g*]isoquinoline-5,12-quinone (**25a**).

The preceding chloroisoquinolinequinone **24** (62 mg, 0.19 mmole) and 3-(diethylamino)propylamine (99 mg, 0.76 mmole) were refluxed in toluene for 5 hours under argon, then the solvent was evaporated. The residue was washed with hexane and recrystallized from cyclohexane, giving 60 mg (75%) of black-purple needles, mp 134°; ^{1}H nmr (deuteriochloroform): δ 1.07 (t, 6H, (CH_3-CH_2)$_2$), 1.88 (m, 2H, β-CH_2), 2.45 (q, 6H, CH_2-CH_3)$_2$ + γ-CH_2), 2.55 (s, 3H, 4-CH_3), 3.69 (q, 2H, α-CH_2), 7.68 (m, 2H, 8-H + 9-H), 7.79 (m, 1H, 10-H), 8.19 (s, 1H, 11-H), 8.43 (s, 1H, 3-H), 8.51 (m, 1H, 7-H), 10.01 (m, 1H, NH), 14.28 (br s, 1H OH).

Anal Calcd. for $C_{25}H_{27}N_3O_3$: C, 71.92; H, 6.52; N, 10.07. Found: C, 72.37; H, 6.64; N, 10.31.

1-[[2-(Dimethylamino)ethyl]amino]-6-hydroxy-4-methylnaphtho-[2,3-*g*]isoquinoline-5,12-quinone (**25b**).

According to the above mentioned technique for **25a** but starting from 2-(dimethylamino)ethylamine, **25b** was obtained after recrystallization from cyclohexane as black purple needles (57%) mp 163°; ^{1}H nmr (deuteriochloroform): δ 2.37 (s, 6H, N(CH_3)$_2$), 2.67 (m, 2H, β-CH_2), 2.70 (s, 3H, 4-CH_3), 3.78 (q, 2H, α-CH_2), 7.79 (m, 2H, 8-H + 9-H), 7.93 (m, 1H, 10-H), 8.25 (s, 1H, 11-H), 8.45 (s, 1H, 3-H), 8.56 (m, 1H, 7-H), 10.07 (br s, 1H, NH), 14.56 (br s, 1H, OH).

Anal. Calcd. for $C_{22}H_{21}N_3O_3 \cdot 0.5C_6H_{12}$: C, 71.92; H, 6.52; N, 10.07. Found: C, 71.49; H, 6.18; N, 9.89.

1-[[2-(2-Hydroxyethyl)aminoethyl]amino]-6-hydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinone (**25c**).

Using 2-[(2-hydroxyethyl)amino]ethylamine and chloroquinone **24** under the conditions described above for compound **25a**, **25c** was recrystallized from ethanol to afford red microcrystals (35%) mp 168°; ^{1}H nmr (deuteriochloroform): [NH-(α)-CH_2-(β)-CH_2-NH-(γ)-CH_2-(δ)-CH_2OH] δ 2.70 (s, 3H, 4-CH_3), 2.97 (m, 4H, β-CH_2 + γ-CH_2), 3.78 (m, 4H, α-CH_2 + δ-CH_2), 7.70 (m, 2H, 8-H + 9-H), 7.94 (m, 1H, 10-H), 8.23 (s, 1H, 11-H), 8.44 (s, 1H, 3-H), 8.52 (m, 1H, 7-H).

Anal. Calcd. for $C_{22}H_{21}N_3O_4 \cdot 0.5C_2H_5OH$: C, 66.65; H, 5.83; N, 10.13. Found: C, 66.95; H, 5.65; N, 9.76.

1-Chloro-6,11-dihydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinone (**27**).

Method A.

A mixture of compound **24** (323 mg, 1 mmole), ammonium nitrate (400 mg, 5 mmoles), trifluoroacetic anhydride (2.9 ml, 20 mmoles) and dry dichloromethane (150 ml) was placed in a flask equipped with a magnetic stirrer, a drying tube and a reflux condenser. The mixture was stirred at room temperature for 2 hours. The solvent was evaporated and the residue was extracted with hot toluene. The extract was washed was water, dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed on a silica gel column, with toluene as eluent. A first pink product was eluted, which was recrystallized from toluene, to give 30 mg (10%) of pink needles, mp 276°, which corresponded to compound **27**; ms: m/z (%) 342 (13.3) 341 (36.7) 340 (26.7) 339 (100); ^{1}H nmr (deuteriochloroform): δ 2.94 (s, 3H, 4-CH_3), 7.86 (m, 2H, 8-H + 9-H), 8.60 (m, 2H, 7-H + 10-H), 8.65 (s, 1H, 3-H), 14.52 + 14.55 (2s, 2H, 6-OH + 11-OH).

Anal. Calcd. for $C_{18}H_{10}ClNO_4$: C, 63.63; H, 2.96; N, 4.12. Found: C, 63.41; H, 2.88; N, 4.05.

Continuing elution with the same solvent, a second compound was isolated, which was recrystallized from toluene to give 21 mg (6%) of red microcrystals, mp >330°. It was identified as being 1-chloro-6-hydroxy-4-methyl-11-nitronaphtho[2,3-*g*]isoquinoline-5,12-quinone **26** ($C_{18}H_9ClN_2O_5$ = 368.5); ms: m/z (%) 371 (9.3), 370 (36), 369 (30.7), 368 (100); ^{1}H nmr (deuteriochloroform): δ 2.90 (s, 3H, 4-CH_3), 7.87 (m, 3H, 7-H + 8-H + 9-H), 8.62 (m, 1H, 10-H), 8.70 (s, 1H, 3-H), 14.54 (s, 1H, OH).

Anal. Calcd. for $C_{18}H_9ClN_2O_5$: C, 58.63; H, 2.46; N, 7.59. Found: C, 58.79; H, 2.39; N, 7.71.

Method B.

Chloroisoquinoline **6** (360 mg, 1.5 mmoles) and benzocyclobutenedione **28** [14,15] (600 mg, 4.5 mmoles) were dissolved in deoxygenated dichloromethane (120 ml). The solution was placed in 35 ml test tubes of 15 mm diameter, then cautiously deareated, stoppered and left at room temperature in the daylight (but not in sunlight) for 5 days [16]. After evaporation of the solvent, the residue was chromatographed as in method A to provide 36 mg (7%) of pure tetracyclic chloroquinone **27**.

1-[[3-(Diethylamino)propyl]amino]-6,11-dihydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinone (**29a**).

This product was obtained as for **25a-c** starting from chloroquinone **27** (23 mg, 0.07 mmole) and 3-(diethylamino)propylamine (35 mg, 0.27 mmole). It was recrystallized from cyclohexane, giving dark purple needles (75% yield) mp 148°; ^{1}H nmr (deuteriochloroform): δ 1.32 (m, 6H, (CH_3 CH_2)$_2$), 2.20 (m, 2H, β-CH_2), 2.66 (s, 3H, 4-CH_3), 2.97 (m, 6H, (CH_2CH_3)$_2$ + γ-CH_2), 3.75 (q, 2H, α-CH_2), 7.77 (m, 2H, 8-H + 9-H), 8.33 (s, 1H, 3-H), 8.43 (m, 2H, 7-H + 10-H), 9.62 (t, 1H, NH), 15.12 + 15.42 (2 br s, 2H, 6-OH + 11-OH).

Anal. Calcd. for $C_{25}H_{27}N_3O_4$: C, 69.26; H, 6.28; N, 9.69. Found: C, 69.00; H, 6.48; N, 9.38.

1-[[2-(Dimethylamino)ethyl]amino]-6,11-dihydroxy-4-methylnaphtho[2,3-*g*]isoquinoline-5,12-quinone (**29b**).

This compound was obtained as its analogue **25b** by using 2-dimethylaminoethylamine and chloroquinone **27**. It was recrystallized from cyclohexane to afford purple needles, mp 208° (75% yield); ^{1}H nmr (deuteriochloroform): δ 2.39 (s, 6H, N(CH_3)$_2$), 2.68 (m, 5H, 4-CH_3 + β-CH_2), 3.76 (q, 2H, α-CH_2), 7.76 (m, 2H, 8-H + 9-H), 8.35 (s, 1H, 3-H), 8.45 (m, 2H, 7-H + 10-H), 9.66 (t, 1H, NH), 15.18 (brs, 2H, 6-OH + 11-OH).

Anal. Calcd. for $C_{22}H_{21}N_3O_4$: C, 67.50; H, 5.41; N, 10.73. Found: C, 67.41; H, 5.75; N, 10.53.

Biological Testing.

In vitro Assay.

Cells (either Friend erythroleukemia C-745 or L1210 and P388 lymphocytic leukemias), from cultures in the late exponential phase of growth (2 x 10^6 c/ml) were seeded in 1 ml microwell plates at 10^5 cells/ml in Dulbecco medium supplemented with 10% fetal calf serum. After 24 hours control cultures were counted (usually 3 to 4 x 10^5 cells/ml); tested compounds were added in duplicate at various concentrations usually between 5 x $10^{-6}M$ and 5 x $10^{-9}M$ and incubated for 24 hours. Cells were counted using a hemocytometer. The IC_{50} were calculated from regression curves obtained with experimental points without significant toxicity and were expressed as the dose inhibiting cell growth by 50%.

In vivo Assay.

P388 leukemia was maintained by serial passages intraperitoneally in DBA/2 mice (10^6 cells i.p.) every 10 days. Experimental determination of antitumor activity was carried out on BDF1 hybrides (6 ♂ + 6 ♀) inocculated with 10^6 cells i.p. at J_0. Test compounds were administred i.p. 24 hours later. Medium life span of untreated mice was 14 ± 1 days. T/C was expressed as:

$$\frac{\text{medium life span of treated mice}}{\text{medium life span of controls}} \times 100$$

T/C > 125 were not significant.

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