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Original paper

Heterocyclic quinones VIII. Synthesis and anti-neoplastic evaluation of 7-substituted-1H-pyrrolo[3,2-c]quinoline-6,9-diones and 3-substituted-11H-indolo[3,2-c]quinoline-1,4-diones *

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Summary — 7-Methoxy-2,3,4-trimethyl-1H-pyrrolo[3,2-c]quinoline-6,9-dione **13** and 3-methoxy-6-methyl-11H-indolo-[3,2-c]quinoline-1,4-diones **15** and **19** were obtained by oxidation of quinolinamines **9**, **11** and **12** with Fremy's salt. The synthesis method of amines **9** and **11** consists of creating the pyrrole or indole nucleus according to Fisher's indolic reaction applied to hydrazones **8** and **10**. Aromatization of **11** yielded quinolinamine **12**. The nucleophilic substitution of methoxy by aziridine leads to quinones **14**, **16** and **20**. These quinones are highly cytotoxic for L1210 cells, but no activity could be established for the P388 lymphocytic leukemia *in vivo*.

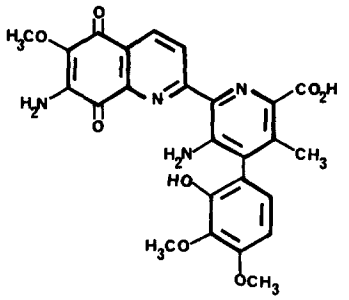
Résumé — **Quinones hétérocycliques VIII. Synthèse et évaluation anti-néoplasique de 1H-pyrrolo[3,2-c]quinoléinediones-6, 9 substituées en 7 et de 1H-indolo[3,2-c]quinoléinediones-1,4 substituées en 3.** *La méthoxy-7 triméthyl-2,3,4 1H-pyrrolo[3,2-c]-quinoléinedione-6, 9* ***13*** *et les méthoxy-3 méthyl-6 11H-indolo[3,2-c]quinoléinediones-1,4* ***15*** *et* ***19*** *sont obtenues par oxydation par le réactif de Frémy des quinolinamines* ***9***, ***11***, ***12*** *correspondantes. La méthode de synthèse des amines* ***9*** *et* ***11*** *consiste à créer le noyau pyrrole ou indole selon la réaction indolique de Fisher appliquée aux hydrazones* ***8*** *et* ***10***. *L'aromatisation de* ***11*** *conduit à la quinoléinamine* ***12***. *La substitution nucléophile du méthoxy par l'aziridine donne les quinones* ***14***, ***16*** *et* ***20***. *Ces quinones sont très cytotoxiques sur les cellules L1210 mais aucune activité n'est constatée sur la leucémie P388* in vivo.

anti-tumor agents / cytotoxicity / Fisher indolic reaction / Fremy's salt / heterocyclic quinones / 11H-indolo[3,2-c]quinoline-1,4-diones / 1H-pyrrolo-[3,2-c]quinoline-6,9-diones / P388 lymphocytic leukemia

Introduction

As described in previous reports [1], the purpose of our work is the study of the heterocyclic quinones related to streptonigrin **1**, whose anti-tumor activity is related to the redox properties of the 5,8-quinolinedione nucleus [2].

Anti-tumor molecules, such as mitomycin [3], have a pyrrole nucleus or a 11H-indolo[3,2-c]quinoline skeleton [4]. According to Marquez *et al.* [4], the 3-chloro-9-diethyl-aminomethyl-8-methoxy-11H-indolo[3,2-c]quinoline **2**, which is devoid of a quinone function, may bind to DNA and inhibit RNA polymerase. More recently, Demarne [5] has described some 11H-indolo[3,2-c]quinolines which



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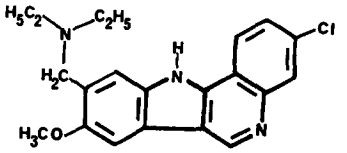

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display *in vivo* anti-tumor activity against Ehrlich's ascitic tumor. It is also worth noting that drugs (netropsin, distamycin [6], CC 1065 [7]) or small synthetic peptide-like oligomers [8] containing pyrrole groups, display sequence specific recognition of B-DNA.

Along these lines, we decided to synthesize new compounds obtained by annelation of the 5,8-quinolinedione nucleus carbons 3 and 4 with the carbons 3 and 2 of the pyrrole or the indole.

This paper describes the synthesis and anti-neoplastic evaluation of 7-substituted-1H-pyrrolo[3,2-c]quinoline-6,9-diones disubstituted in C_2 and C_3 positions by two methyl groups **13**, **14** or by a tetramethylene chain **15**, **16** and **17** and of the 3-substituted 11H-indolo[3,2-c]quinoline-1,4-diones **19** and **20**.

Chemistry

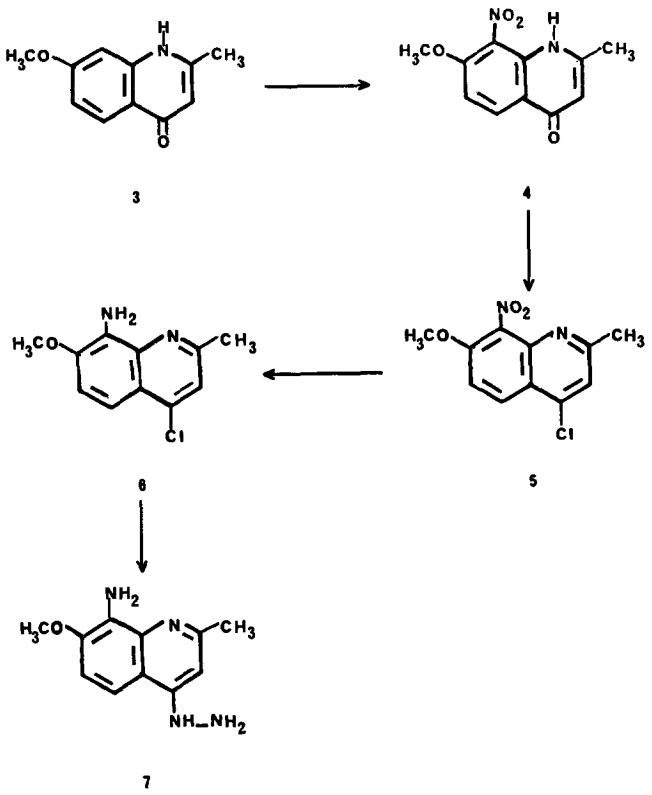
Because of their high reactivity, the quinone functions were introduced at the last stage of the synthesis. Moreover, various synthetic methods which are likely to lead to the 1H-pyrrolo[3,2-c]quinolines and to the 11H-indolo-[3,2-c]quinolines were studied [9].

The syntheses are indicated in Schemes 1, 2 and 3. The 7-methoxy-2-methyl-4(1H)-quinolinone **3** is selectively nitrated on the C_8 position by potassium nitrate in sulfuric acid to give compound **4** (Scheme 1). Its structure was established by NMR (*ortho* coupling between protons H_5 and H_6). The orientation of the electrophilic substitution in C_8 position by the influence of the methoxy group in C_7 is similar to that described by Heindel *et al.* [10] for the 4,7-dichloroquinoline nitration.

The chlorination of the 4(1H)-quinolinone **4** gave the corresponding chloroquinoline **5**. The reduction of the nitro groups of the 4-chloro-7-methoxy-2-methyl-8-nitroquinoline **5**, without hydrogenolysis of the carbon—chlorine bond, is achieved in the presence of Raney's nickel yielding 94% of the corresponding amine **6**. This is higher than the 50% yield obtained when reduction was performed with iron in an acetic acid medium according to Price's method [11] for 4-chloro-6-methoxy-8-nitroquinoline.

The synthesis of the 4-hydrazino-7-methoxy-2-methyl-8-quinolinamine **7** is difficult because of the instability of the hydrazine function in an ethanolic solution, even in the cold. When excess hydrazinium hydroxide without solvent was reacted with **6**, **7** was obtained in 77% yield.

The hydrazones **8** and **10** can be synthesized in the reaction of hydrazine **7** with butanone or cyclohexanone, respectively (Scheme 2). Amines **9** and **11** are obtained by applying Fischer's indolic reaction [12] to hydrazones **8** and **10**.



3 4 5 6 7

Scheme 1.

The aromatization of amine **11**, by catalytic dehydrogenation in the presence of palladium on carbon in boiling decalin leading to the 3-methoxy-6-methyl-11H-indolo [3,2-c]quinoline-4-amine **12**, is an original procedure for the synthesis of this heterocyclic compound (Scheme 2).

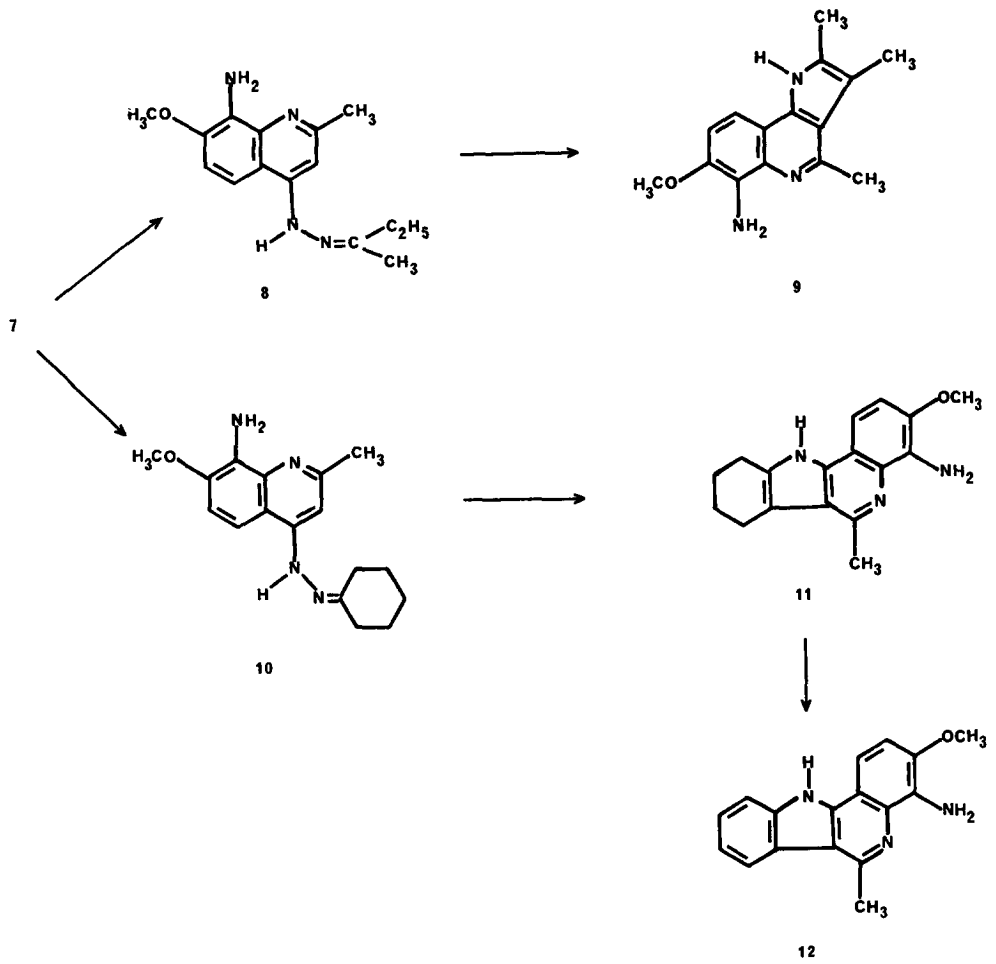
An easy synthetic route to quinones **13**, **15** and **19** is described (Scheme 3). These quinones are prepared by oxidation of the corresponding amines **9**, **11** and **12** using Fremy's salt [13] (potassium nitrosodisulfonate). Crude quinones are obtained along with quinonimines which cannot be isolated in a pure state and are quickly hydrolyzed into quinones by an acid treatment. For example, amine **12** leads to a 15—85% mixture of quinonimine **18** and quinone **19**, respectively (NMR quantitative analysis).

Quinones **13**, **15** and **19** are sensitive to nucleophilic substitution (Scheme 3). Due to its ester-like properties, the methoxy group can be replaced by an amino group and particularly by an aziridine group. This last point is interesting because of the anti-tumor action of many (1-aziridinyl)-*p*-quinones such as AZQ or NSC-182986 [14, 15]. Quinones **14**, **16** and **20** are obtained in fair yields when prepared in methanol with a large excess of amine.

Pharmacology

Cytotoxic and anti-tumor activities

The compounds were tested on L1210 cells *in vitro* and the P388 leukemia *in vivo*. The concentrations that inhibit



Scheme 2.

Table I. Cytotoxic effects of 1H-pyrrolo[3,2-c]quinoline-6,9-diones and 11H-indolo[3,2-c]quinoline-1,4-diones on the growth of L1210 cells *in vitro*.

Compound	IC_{50}[a] 48 h	
	ng/ml	10^{-6} M
13	543	2.01
15	867	2.57
19	501	1.62
14	39	0.132
16	60	0.189
17	3164	9.07
20	65	0.186
Doxorubicin[b]	28	0.048

[a] IC_{50} was determined by a least-squares plotting of the experimental data. The correlation coefficient in all cases was above 0.99.
[b] Doxorubicin was used as a reference compound.

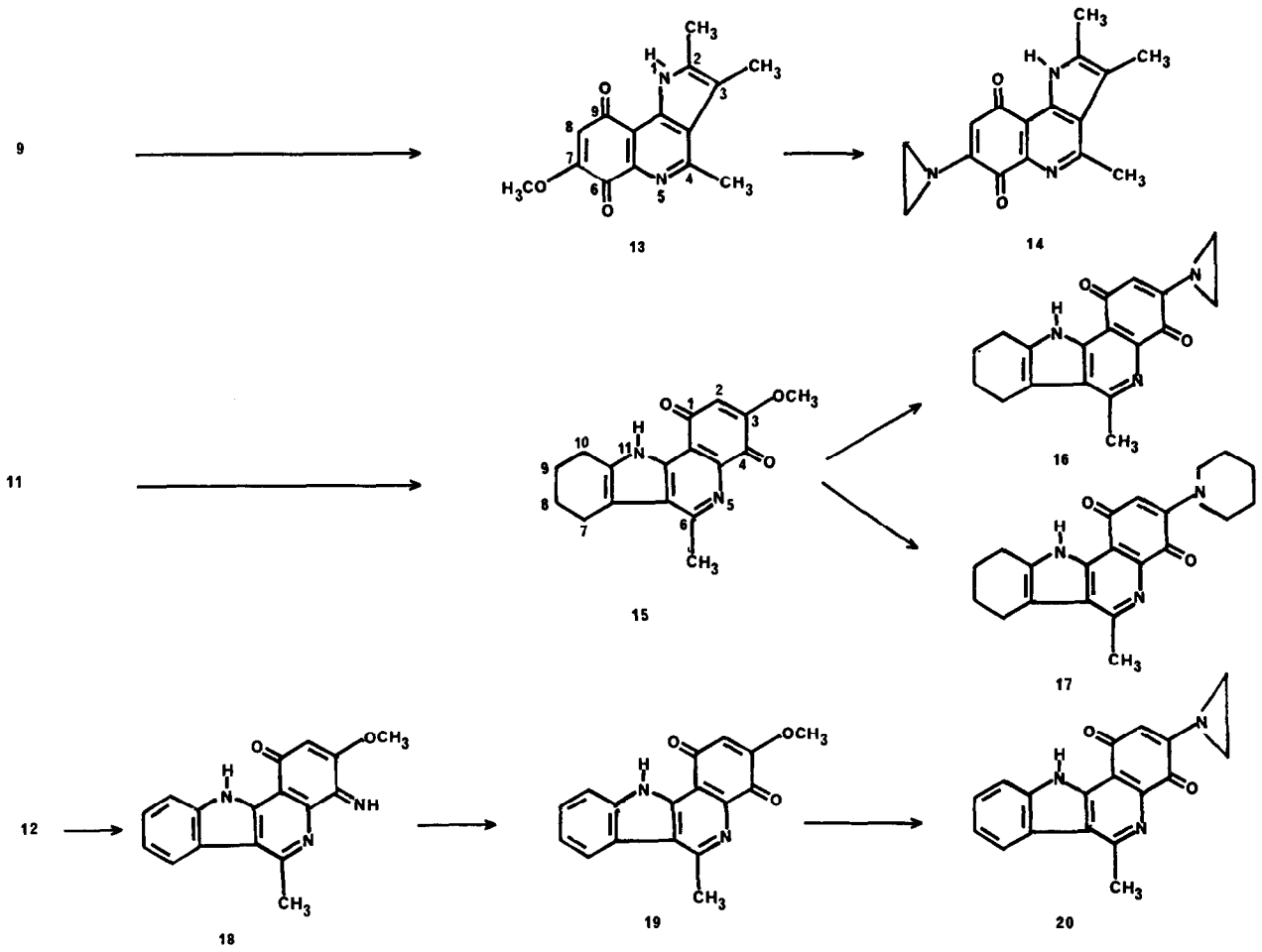
the growth of L1210 cells to 50% of control growth (IC_{50}) after 48 h of culture are indicated in Table I. Three compounds have an IC_{50} lower than 1 μM: **14** (0.13), **16** (0.19) and **20** (0.19).

These quinones were selected for *in vivo* testing on lymphocytic leukemia P388 (Table II). Anti-tumor activity was expressed as $T/C \times 100$, T being the median survival time of treated animals and C being the median survival time of control animals. No significant activity was noted on P388 leukemia under our experimental conditions ($T/C < 125\%$).

Discussion

Using a tetramethylene link, in quinone **15** and aziridinoquinone **16**, as a substitute for methyl groups at C_2 and C_3 of the 1H-pyrrolo[3,2-c]quinoline-6,9-diones **13** and **14** leads to a weak lowering of cytotoxicity. On the other end, the aromatization of the 7,8,9,10-tetrahydro-11H-indolo[3,2-c]quinoline-1,4-dione **15** to give **19** has a weak favorable effect.

The aromatization does not modify the cytotoxicity of aziridinoquinones: compare **16** with **20**. The nucleophilic substitution of the methoxy group in **15** by a piperidinyl group, **17**, considerably reduces cytotoxicity. If the



Scheme 3.

Table II. Effect of compounds **16, 14, 20** on P388 lymphocytic leukemia[a].

Reference compound[b]	Treatment		Dose level (mg/kg/inj.)	Toxicity day survivors[c]	Body weight change (g) (D_5—D_1)	Median survival time (day)	T/C (%)[d]
	Route	Schedule					
16	ip	QO_1DXO1	30	06/10	— 2.5	8.7	107
			25	10/10	— 1.5	8.1	100
			20	10/10	— 1.5	8.1	100
Control (16)					+ 2.0	8.1	—
16	ip	QO_4DXO3	20	10/10	— 1.4	11.8	117
		QO_4DXO3	10	10/10	+ 0.2	11.9	118
		QO_4DXO3	5	10/10	+ 0.9	11.6	115
Control (10)			—	—	+ 2.2	10.1	—
14	ip	QO_1DXO1	25	08/10	— 3.6	11.3	111
		QO_1DXO1	12.5	10/10	— 1.2	10.4	102
		QO_1DXO1	6.25	10/10	— 0.1	11.1	109
20	ip	QO_1DXO1	100	09/10	+ 2.7	10.1	99
Control (18)		—	—	—	+ 1.8	10.2	—

[a]P388 cells (10^6) were inoculated ip on day 0 into CD_2F_1 mice.
[b]( ) Number of animals tested.
[c]Number of survivors on day 5/number of treated mice.
[d]Ratio of median survival time of treated animals (T) to median survival time of control (C) expressed as a percentage.

secondary amine is aziridine, we get quinones **14**, **16** and **20** which are more cytotoxic. The increase of cytotoxicity is very likely due to the alkylating action of this 1-aziridinyl group that we find with AZQ [14, 15] (bisaziridinylbenzoquinone). Unfortunately, the compounds **14**, **16** and **20** are inactive *in vivo* on the P388 lymphocytic leukemia.

Hence, the annelation of the 5,8-quinolinedione nucleus by its carbons 3 and 4 with the carbons 3 and 2 of the pyrrole or the indole leads to molecules cytotoxic for L1210 leukemia cells when a methoxy group is in the position similar to position 7 of quinoline. These compounds are cytotoxic when an aziridinyl group serves as a substitute for the methoxy group. They are inactive *in vivo* on P388 lymphocytic leukemia. We have no explanation for the lack of activity. The IC_{50} values for L1210 cells *in vitro* suggest that these compounds are not particularly cytotoxic. In addition, metabolic inactivation might be a factor in the lack of *in vivo* activity [18]. The influence of the methoxy group in position 6 of quinoline, a position similar to that of the methoxy of streptonigrin **1**, on the biological activity will be studied later.

Experimental protocols

Chemistry

Melting points were determined on a Maquenne apparatus and are uncorrected. Infrared spectra were obtained on a Perkin—Elmer Model 157G spectrometer (KBr) cm^{-1}. NMR spectra were recorded on a Bruker 270 MHz spectrometer with $(Me_3Si)_2$ as an internal standard. Thin—layer chromatography was carried out using Merck GF 254 silica gel. All elemental analyses were within 0.4% of the theoretical values.

7-Methoxy-2-methyl-8-nitro-4(1H)-quinolinone **4**

To 5.7 g (30 mmol) of 7-methoxy-2-methyl-4(1H)-quinolinone **3** [16] dissolved in 50 ml of sulfuric acid (*d* 1.84) were added 3.1 g (30.6 mmol) of potassium nitrate. The mixture was stirred at 0°C for 3 h before being poured onto ice; ammonia (*d* 0.9) was added while cooling until a pH of 7 was reached. The solid that separated was filtered, washed with H_2O, dried and recrystallized from acetone. Yield 81% (5.7 g). mp = 249°C. Anal. ($C_{11}H_{10}N_2O_4$), C, H, N. IR CH_3O 1100, νNO_2 1350 and 1505, $\nu C{=}O$ 1660, νNH 3380. NMR (Me_2SO-d_6) δ: 2.3 (s, 3, CH_3); 3.9 (s, 3, CH_3O); 5.8 (s, 1, H_3); 7.2 (d, 1, $J \simeq 9$ Hz, H_6); 8.05 (d, 1, H_7); 10.85 (s, 1, NH).

4-Chloro-7-methoxy-2-methyl-8-nitroquinoline **5**

A solution of 4.68 g (20 mmol) of **4** in 20 ml of $POCl_3$ was stirred at 105°C for 4 h. The excess $POCl_3$ was evaporated *in vacuo* and the residue poured into a mixture of iced $CHCl_3$—ammonia. The solvent layer was washed with water, dried, treated with charcoal and eliminated *in vacuo*. After crystallization from butanol, compound **5** melted at 174°C. Yield 88% (4.5 g). Anal. ($C_{11}H_9ClN_2O_3$) C, H, N. IR CH_3O 1110, νNO_2 1380 and 1540. NMR (Me_2SO-d_6) δ: 2.55 (s, 3, CH_3); 4.0 (s, 3, CH_3O); 7.5 (s, 1, H_3); 7.6 (d, 1, $J \simeq 9$ Hz, H_6); 8.1 (d, 1, H_5).

4-Chloro-7-methoxy-2-methyl-8-quinolinamine **6**

A solution of 5.05 g (20 mmol) of **5** in 200 ml of a 1:2 mixture of MeOH and dioxane was hydrogenated under catalysis of 5 g of Raney's nickel until the gas uptake ceased (60.4 mmol). After filtration from catalyst under N_2, the mixture was charcoaled and evaporated to give 4.2 g (94%) of **6** after crystallization from a 1:1 mixture of EtOH—H_2O: mp = 120°C. Anal. ($C_{11}H_{11}ClN_2O$) C, H, N. IR: CH_3O 1105, νNH_2 3380 and 3440. NMR (Me_2SO-d_6) δ: 2.5 (s, 3, CH_3); 3.8 (s, 3, CH_3O); 5.2 (s, 2, NH_2); 7.15 and 7.30 (d, 1, $J \simeq 9$ Hz, H_5 and H_6); 7.3 (s, 1, H_3).

4-Hydrazino-7-methoxy-2-methyl-8-quinolinamine **7**

A suspension of 4 g (18 mmol) of **6** in 150 ml of a hydrazinium hydroxide was heated at 110°C under N_2 for 3 h. The precipitate was filtered, washed with H_2O, dried and recrystallized from 1:1 MeOH—H_2O to give 3 g (77%) of essentially pure **7**: mp = 169°C. Anal. ($C_{11}H_{14}N_4O$) C, H, N. IR CH_3O 1080, νNH_2 3330 and 3435. NMR (Me_2SO-d_6) δ: 2.4 (s, 3, CH_3); 3.75 (s, 3, CH_3O); 4.15 and 4.9 (s, 2, NH_2); 6.55 (s, 1, H_3); 6.95 and 7.2 (d, 1, $J \simeq 9$ Hz, H_7 and H_8), 8.0 (s, 1, NH).

7-Methoxy-2,3,4-trimethyl-1H-pyrrolo[3,2-c]quinoline-6-amine **9**

Compound **7** (1.3 g, 6 mmol) in 10 ml of EtOH and 1.6 ml (18 mmol) of butanone were heated under N_2 for 3 h. The solvent was evaporated *in vacuo* to give the hydrazone **8** (1.47 g). The hydrazone (1.47 g, 5.4 mmol), without purification, was taken up in 10 ml of diethylene glycol and heated at 250°C under N_2 for 1 h. The reaction mixture was cooled and diluted with 50 ml of water. The precipitate was filtered, washed with water to remove the solvent, dried and then crystallized from aqueous ethanol 1.2 g (84%). mp = 196°C. Anal. ($C_{15}H_{17}N_3O$, 1.25 H_2O) C, H, N. IR CH_3O 1085, νNH 3200, νNH_2 3330 and 3410. NMR (Me_2SO-d_6) δ: 2.3 (s, 6, Me-2 and Me-3); 2.75 (s, 3, Me-4); 3.8 (s, 3, CH_3O); 5.05 (s, 2, NH_2); 7.1 and 7.35 (d, 1, $J \simeq 9$ Hz, H_8 and H_9); 11.55 (s, 1, NH).

3-Methoxy-6-methyl-7,8,9,10-tetrahydro-11H-indolo[3,2-c]quinoline-4-amine **11**

Cyclohexanone (1.6 ml, 15.5 mmol), hydrated sodium acetate (3.8 g, 28 mmol) and **7** (3.05 g, 14 mmol) were added to 40% aqueous acetic acid (30 ml), which was boiled under N_2 for 30 min, cooled and poured into dilute ammonia (*d* 0.9). The precipitated solid **10** was collected and washed with water. Compound **11** was obtained from the cyclization of hydrazone **10**, without purification, in the same manner as **9**. After crystallization from benzene, compound **11** melted at 218°C. Yield 88% (3.46 g). Anal. ($C_{17}H_{19}N_3O$) C, H, N. IR CH_3O 1090, νNH 3200, νNH_2 3350 and 3430. NMR (Me_2SO-d_6) δ: 1.75 (m, 4, $2CH_2$); 2.7 (m, 5, CH_2 and CH_3); 2.85 (m, 2, CH_2); 3.8 (s, 3, CH_3O); 5.05 (s, 2, NH_2); 7.1 and 7.3 (d, 1, $J \simeq 9$ Hz, H_1 and H_2); 11.55 (s, 1, NH).

3-Methoxy-6-methyl-11H-indolo[3,2-c]quinoline-4-amine **12**

A suspension of 1.13 g (4 mmol) of **11** and 1.2 g of 10% Pd on activated charcoal in 25 ml of decalin was refluxed under N_2 for 10 h. After cooling, the solid was filtered and then extracted with $CHCl_3$—MeOH (1:1), dried and evaporated. After crystallization from EtOH—H_2O, compound **12** melted at 247°C. Yield 57% (0.67 g). Anal. ($C_{17}H_{15}N_3O$, H_2O) C, H, N. IR CH_3O 1080, νNH 3170, νNH_2 3320 and 3410. NMR (Me_2SO-d_6) δ: 3.0 (s, 3, CH_3), 3.9 (s, 3, CH_3O); 5.25 (s, 2, NH_2); 7.3 (d and t, 2, $J_{8-9} \simeq 8$ Hz, H_2 and H_3); 7.4 (t, 1, $J_{9-10} \simeq 8$ Hz, H_9); 7.6 (d and d, 2, $J_{1-2} \simeq 9$ Hz, H_1 and H_{10}); 8.1 (d, 1, $J_{7-8} \simeq 8$ Hz, H_7); 12.4 (s, 1, NH).

General procedure for the synthesis of ***13***, ***15*** *and* ***19***

A solution of 2.72 g (20 mmol) of monobasic potassium phosphate in *x* ml of water was added to 4 mmol of amino compound in *y* ml of solvent. To this mixture which was vigorously stirred at ambient temperature were added 4.3 g (16 mmol) of potassium nitrosodisulfonate for 15 min. The mixture was stirred for *m* h. Hydrochloric acid (2 M) (*z* ml) was added and was stirred for an additionnal *n* h, sodium hydrogenocarbonate was added until a pH of 7 was reached. The organic solvent was eliminated *in vacuo*, the residue was dissolved in dichloromethane, washed with H_2O, dried and evaporated to dryness to give a pyrroloquinoline-6,9-dione. The amine **12** was oxidized by potassium nitrosodisulfonate to give a precipitate of indoloquinoline-1,4-dione. The precipitate was filtered and then extracted with 1:1 $CHCl_3$—MeOH, dried, charcoaled and evaporated.

7-Methoxy-2,3,4-trimethyl-1H-pyrrolo[3,2-c]quinoline-6,9-dione **13**. x = 40, y = 80, acetone, m = 3, z = 5, n = 2. Recrystallized from MeOH. Yield 42%. mp = 287°C. Anal. ($C_{15}H_{14}N_2O_3$) C, H, N. IR $\nu C{=}O$ 1630 and 1680, νNH 3350. NMR (Me_2SO-d_6) δ: 2.3 and 2.35 (s, 6, Me-2 and Me-3); 2.75 (s, 3, Me-4); 3.8 (s, 3, CH_3O); 6.1 (s, 1, H_8); 11.65 (s, 1, NH).

3-Methoxy-6-methyl-7,8,9,10-tetrahydro-11H-indolo[3,2-c]quinoline-1,4-dione **15**. x = 85, y = 140, MeOH—acetone (1:1), m = 3, z = 6, n = 2. Recrystallized from acetonitrile. Yield 80%. mp = 295°C.

Anal. ($C_{17}H_{16}N_2O_3$, CH_3CN) C, H, N. IR CH_3O 1100, $\nu C{=}O$ 1635 and 1685, νNH 3300. NMR (Me_2SO-d_6) δ: 1.75 (m, 4, $2CH_2$); 2.7 (m, 5, CH_2 and CH_3); 2.8 (m, 2, CH_2); 3.8 (s, 3, CH_3O); 6.1 (s, 1, H_2); 11.7 (s, 1, NH).

3-Methoxy-6-methyl-11H-indolo[3,2-c]quinoline-1,4-dione ***19***. x = 40, y = 160, MeOH—acetone (1:1), m = 9, z = 6, n = 4. Recrystallized from MeOH. Yield 35%. mp = 314°C. Anal. ($C_{17}H_{12}N_2O_3$, H_2O) C, H, N. IR $\nu C{=}O$ 1630 and 1690, νNH 3360 and 3600. NMR (Me_2SO-d_6) δ: 3.0 (s, 3, CH_3); 3.8 (s, 3, CH_3O); 6.2 (s, 1, H_2); 7.3 (t, 1, $J_{8-9} \simeq$ 8 Hz, H_8); 7.5 (t, 1, $J_{9-10} \simeq$ 8 Hz, H_9); 7.8 (d, 1, H_{10}) 8.15 (d, 1, $J_{7-8} \simeq$ 8 Hz, H_7); 12.25 (s, 1, NH).

General procedure for synthesis of ***14***, ***16*** *and* ***20***

To a suspension of 0.75 mmol of quinone **13**, **15** or **19** in 7.5 ml of dry MeOH, was added 3.1 ml (60 mmol) of aziridine. The mixture was stirred at 20°C for x h. The solid that separated was filtered, washed with petroleum ether and recrystallized from MeOH.

7-(1-Aziridinyl)-2,3,4-trimethyl-1H-pyrrolo[3,2-c]quinoline-6,9-dione ***14***. x = 4. Yield 51%. mp = 217°C. Anal. ($C_{16}H_{15}N_3O_2$, 0.75 H_2O) C, H, N. IR $\nu C{=}O$ 1630 and 1670, νNH 3320 and 3440. NMR (Me_2SO-d_6) δ: 2.2 (m, 4, $2CH_2$); 2.3 and 2.35 (s, 6, Me-2 and Me-3); 2.75 (s, 3, Me-4); 6.1 (s, 1, H_8); 11.55 (s, 1, NH).

3-(1-Aziridinyl)-6-methyl-7,8,9,10-tetrahydro-11H-indolo[3,2-c]quinoline-1,4-dione ***16***. x = 2. Yield 50%. mp = 200°C. Anal. ($C_{18}H_{17}N_3O_2$, 0.5 H_2O) C, H, N. IR $\nu C{=}O$ 1620 and 1675, νNH 3340 and 3420. NMR (Me_2SO-d_6) δ: 1.75 (m, 4, 2 CH_2), 2.2 (m, 4, N—CH_2); 2.75 (m, 5, CH_2 and CH_3); 2.85 (m, 2, CH_2); 6.15 (s, 1, H_2); 11.65 (s, 1, NH).

3-(1-Aziridinyl)-6-methyl-11H-indolo[3,2-c]quinoline-1,4-dione ***20***. x = 5. Yield 43%. mp = 215°C. Anal. ($C_{18}H_{13}N_3O_2$, 2.5 H_2O) C, H, N. IR $\nu C{=}O$ 1630 and 1675, νNH 3420. NMR (Me_2SO-d_6) δ: 2.25 (m, 4, 2 CH_2); 3.0 (s, 3, CH_3); 6.25 (s, 1, H_2); 7.3 (t, 1, $J_{8-9} \simeq$ 8 Hz, H_8); 7.5 (t, 1, $J_{9-10} \simeq$ 8 Hz, H_9), 7.8 (d, 1, H_{10}), 8.15 (d, 1, $J_{7-8} \simeq$ 8 Hz, H_7); 12.2 (s, 1, NH).

6-Methyl-3-piperidino-7,8,9,10-tetrahydro-11H-indolo[3,2-c]quinoline-1,4-dione ***17***

To a suspension of 0.22 g (0.75 mmol) of **15** in 5 ml of dry MeOH was added 7.4 ml (75 mmol) of piperidine. The mixture was stirred at 20°C for 24 h. The solid precipitate **17** was collected, washed and then purified on preparative alumina plate (solvent: $CHCl_3$). Yield 67%. mp = 264°C. Anal. ($C_{21}H_{23}N_3O_2$) C, H, N. IR $\nu C{=}O$ 1605 and 1670, νNH 3280. NMR (Me_2SO-d_6) δ: 1.6 (m, 6, 3 CH_2); 1.75 (m, 4, 2 CH_2); 2.7 (m, 5, CH_2 and CH_3); 2.85 (m, 2, CH_2); 3.4 (m, 4, 2 CH_2); 5.75 (s, 1, H_2); 11.6 (s, 1, NH).

Pharmacology

Growth inhibition of L1210 cells in culture

The experimental protocol has previously been reported [17]. The cells were exposed to increasing concentrations of drugs dissolved in Me_2SO (1% final concentration) and incubated at 37°C without agitation in a 5% CO_2 atmosphere.

Cells either in the presence of the heterocyclic quinones or in their absence (control) were counted in triplicate after 24 and 48 h of culture with a Coultronics Coulter Counter. The cytotoxic activity of the compounds was measured by determining the drug concentration which decreased the growth rate of L1210 cells to 50% of that of the control cells. The IC_{50} was estimated from equations obtained by plotting the logarithm of the drug concentration *versus* the probit of the percentage of the inhibition of growth.

Anti-tumor activity

The compounds were studied on P388 lymphocytic leukemia. Experiments were performed on 2 month old CD_2F_1 mice (Balb/c × DBA/2).

The anti-tumor tests were performed using the protocols and evaluation criteria established by the NCI. P388 leukemia cells (10^6) were injected ip into mice on day 0. Treatments were given ip on day 1 (compounds **14**, **16** and **20**) or days 1, 5 and 9 (compound **16**).

The drugs were inoculated as suspensions in 0.4% hydroxypropylcellulose (J. F. Klucel, Hercules Co.) solution in water. The mortality was checked daily for 30 days. Anti-tumor activity was expressed as $T/C \times 100$, T being the median survival time of treated animals and C being the median survival time of control animals. The median survival time is obtained from dead animals only excluding cures from calculation.

Anti-tumor activity is considered significant when $T/C \times 100 \geqslant 125\%$. A T/C value $\leqslant 85\%$ indicates a toxic test.

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